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Seasonal Metacommunity Processes of Benthic Invertebrates in the Vjosa/Aoos River

Jan Martini[1,2,3] | Thomas Fuß[1] | Marie V. Brasseur[4] | Rebecca Oester[5,6,7] | Lauren Talluto[1,8] | Franziska Walther[9] | Olivia Wilfling[10] | Vera M. A. Zizka[11] | Gabriel Singer[1] | Simon Vitecek[1,3,10,12]

[1]Department of Ecology, University of Innsbruck, Innsbruck, Austria | [2]gutwasser GmbH, Zurich, Switzerland | [3]WasserCluster Lunz—Biologische Station GmbH, Lunz am See, Austria | [4]Ecotoxicology, University Duisburg-Essen, Essen, Germany | [5]Institute of Microbiology, University of Applied Sciences and Arts of Southern Switzerland, Mendrisio, Switzerland | [6]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland | [7]Eawag: Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, Dübendorf, Switzerland | [8]International Institute of Applied Systems Analysis, Biodiversity, Ecology, and Conservation Group, Laxenburg, Austria | [9]Chair of Planning of Landscape and Urban Systems (PLUS), Institute for Spatial and Landscape Development, ETH Zurich, Zurich, Switzerland | [10]Institute of Hydrobiology and Aquatic Ecosystem Management, University of Natural Resources and Life Sciences, Vienna, Austria | [11]State Museum of Natural History Karlsruhe, Karlsruhe, Germany | [12]Senckenberg Research Institute and Natural History Museum, Frankfurt am Main, Germany

Correspondence: Jan Martini (elvanjan@gmail.com) | Simon Vitecek (simon.vitecek@boku.ac.at)

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ABSTRACT

There are only a handful of free-flowing rivers left on our planet. The unique biodiversity in these model systems offers a key to understanding the structures of naturally assembled fluvial metacommunities. Here, we use the benthic invertebrate fauna of the Vjosa River as a model system to infer metacommunity processes from biodiversity patterns at river network scale. To this end, we used data from standard, morphology-based and metabarcoding approaches to describe community composition at 36 sites in spring and autumn. Classical ordination as well as joint species distribution modeling indicate that community composition in headwaters is mostly defined by (likely limited) dispersal and environmental filtering, whereas communities at downstream sites in the main stem are shaped more by associations among taxa. At the population level of individual taxa, the relative importance of spatial factors, environmental filtering, and potential associations to other taxa changed between seasons. Our study provides a realistic impression of the ecology of fluvial metacommunities under the fluctuating influences of spatial structure, environmental conditions, and biotic interactions in a free-flowing river network.

1 | Introduction

Life is ubiquitous on our planet, but not uniformly distributed. At large spatial scales, environmental heterogeneity and evolutionary background shape regional species pools (Ricklefs 1987; Carstensen et al. 2013; Cornell and Harrison 2014), from which local communities assemble at patch-scale through environmental filtering in dependence of dispersal (Loreau et al. 2003; Gravel et al. 2010; Cornell and Harrison 2014). Intermediate dispersal facilitates patch-scale filtering most efficiently (MacArthur and Wilson 1963; Vanschoenwinkel et al. 2007; Heino et al. 2015) allowing colonization of habitats by suitable species, resulting in a community matching the environment well and supporting high biodiversity and productivity (Venail et al. 2008; Yamaguchi 2022; Fuß et al. 2024, 2025). In contrast, we observe a community-environment mismatch in (i) isolated patches, where low dispersal (i.e., insurmountable space) causes suitable habitats to remain unoccupied, thus creating a colonization credit, and (ii) overly well-connected patches, where high dispersal (i.e., space is easily surmountable) allows strong mass effects and enables persistence under unsuitable conditions (Jacobson and Peres-Neto 2010; Talluto et al. 2017). Dispersal mechanisms are integrated in the

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metacommunity concept which describes the dynamic turnover of multiple, connected local communities as a consequence of environmental conditions and the distance among patches that determine colonization/extinction dynamics (MacArthur and Wilson 1963; Leibold et al. 2004; Brown et al. 2011). At the level of individual species, biotic interactions (positive and negative) must be understood as integral niche components and thus influence the composition of local communities as well (Wisz et al. 2013; Singer et al. 2013; Godsoe et al. 2017; Stephan et al. 2021).

In this context, rivers and streams present a special case by constraining metacommunity processes through their hierarchical and dendritic spatial structure. Rivers drain geologically complex landscapes and thus create unique network topologies as distinct landforms (Brown and Swan 2010; Altermatt 2013). Larger river sections with distinct environmental properties form sequentially along the river network depending on the geological background, slope, and discharge (Hauer et al. 2021). The spatial topology of the river network shapes the dynamics of discharge (Widder et al.), which drive disturbance regimes that shape local habitat patches (Hauer et al. 2012), and control primary dispersal routes for lotic biota (Tonkin et al. 2018; Talluto et al. 2024). In consequence, spatial properties of river networks can be expected to manifest in specific metacommunity structures—where the importance of space, environment, and biotic interactions varies along/across the river network (Talluto et al. 2024; Fuß et al. 2025), thus bringing about the disproportionately high biodiversity of lotic freshwaters (Tickner et al. 2020; Albert et al. 2021).

Notably, the delicate riverine metacommunity structure can easily be disrupted: locally through habitat modification (e.g., by bank or sole fortification) and regionally through modification of water flow, for example, by damming or water abstraction affecting connectivity (Vörösmarty et al. 2010). In the global biodiversity crisis, understanding the drivers of riverine metacommunity structure is key to designing effective conservation, protection and restoration strategies (Cid et al. 2022; Stoffers et al. 2024; Keck et al. 2025).

Controls of metacommunity processes are typically analyzed by assessing the importance of sets of variables that describe effects of space, environment and associations among taxa on β-diversity patterns using ordination-based analysis (Warton et al. 2015). Statistical inference of metacommunity processes is not trivial and usually yields high residual variation that cannot be linked to effects of neither space nor environment nor associations among taxa. Recently, joint species distribution models (jSDMs) were developed to include latent variables to model residual covariance as a proxy for associations among taxa (Leibold et al. 2022).

Assessing spatial, environmental and biotic variables of riverine metacommunities at a relevant resolution is especially challenging: While automated collection of environmental parameters and better approaches to describe the spatial structure of a river are now available, assessing biodiversity at the relevant scale of the entire river network remains a challenge. This is because of difficulties associated with representative sampling of the riverine biota but also because critical taxonomic knowledge and resources needed for identification are often not available (Heino 2014). Here, DNA metabarcoding offers an alternative promising means to assess biodiversity (Brasseur et al. 2023).

In light of these methodological developments and the critical need for a better understanding of factors that underlie the assembly of riverine metacommunities, we use the Vjosa River as a model ecosystem that spotlights urgent conservation needs and the opportunity to analyze natural metacommunity processes. The Vjosa River is the last river network in Europe retaining a near-natural character; it is a refuge of European significance for almost extinct riverine fauna and flora (Schiemer et al. 2020). Rare fauna such as *Prosopistoma pennigerum* (Martini et al. 2023; Schwingshackl et al. 2024) or *Thremma anomalum* (Waringer et al. 2020) and recently discovered endemic species (*Isoperla vjosae*, Graf et al. 2018) highlight the significance of the Vjosa River as a conservation target and a restoration model (Schiemer et al. 2020). Investigating the metacommunity structure of benthic invertebrate fauna is particularly relevant, as this group is extraordinarily diverse, highly sensitive and a legally recognized biological quality element for assessing a river's ecological integrity (Birk et al. 2012; Bonada et al. 2006; Hering et al. 2010). Developing a better understanding of the drivers of benthic invertebrate metacommunity structure in a near-natural model system like the Vjosa River may help design biodiversity targets for European rivers at large scales (Schiemer et al. 2020; Stoffers et al. 2024). An important question in this context is how much can be learned about the riverine metacommunity structure from different types of biodiversity data—standard taxonomic or DNA metabarcoding approaches—and whether any uncovered patterns are seasonally comparable. In particular, the importance of individual determinants of ecological processes might change with seasonally structured connectivity, environmental controls (Poff et al. 1997; Datry et al. 2014; Tonkin et al. 2018) or ontogenetic development of benthic invertebrates (Ward 1985; Durance and Ormerod 2007; Nakazawa 2015).

Here, we explicitly address this issue by inferring the relative importance of metacommunity processes in structuring benthic invertebrate fauna over the spatial and environmental extent of the Vjosa River network at two different timepoints based on standard, morphology-based and DNA metabarcoding-derived biodiversity data. Our hypotheses acknowledge the hierarchical structure of the Vjosa stream network and posit (i) highly divergent headwater tributary community composition as determined by space and environment because of their isolation at the extremity of environmental gradients in diverse geological settings; (ii) mid-stream reaches and larger mainstem tributaries' community composition is regulated by intermediate isolation and high environmental heterogeneity as well as associations of taxa; and (iii) mainstem community composition under the rule of space and associations among taxa in the environmentally homogeneous and maximally connected downstream reaches of the Vjosa River.

2 | Methods

2.1 | Sampling Sites

The Vjosa River network integrates streams and rivers in a catchment of over 6700 km^2 from the Pindos Mountains to the Adriatic Sea. We sampled 45 sites across the network (Figure 1) concentrated around confluences where two sites were sampled in each tributary and one in the resulting stream, in April–May and October 2018. We excluded 9 sites due to being dry in autumn ($n = 2$), missing molecular data

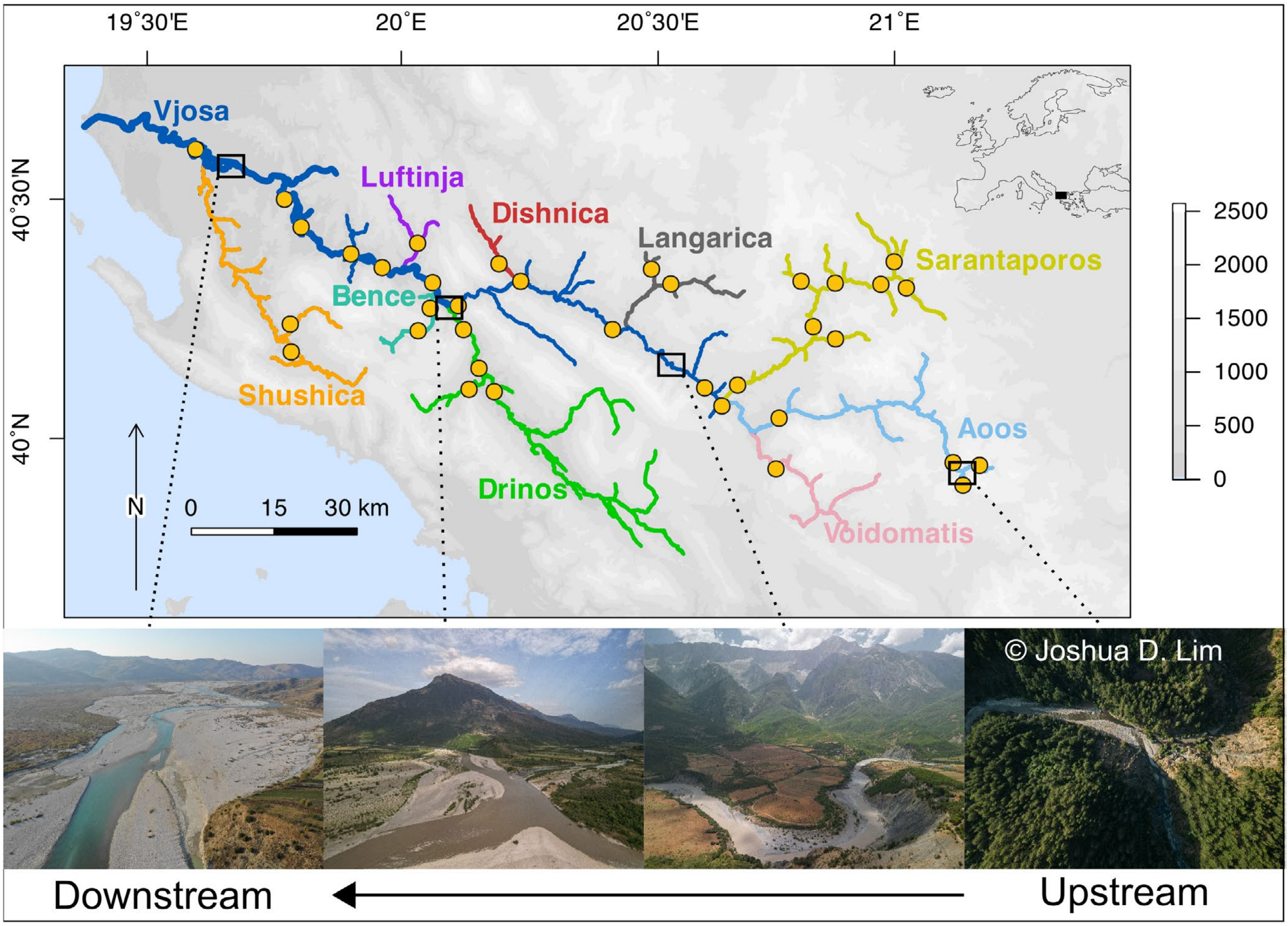

FIGURE 1 | Spring and autumn 2018 sampling sites (36) along the Vjosa-Aoos River (light and dark blue) and its major tributaries (various colors). Landscape pictures show four exemplary sections illustrating the environmental gradient along/across the river network.

($n = 3$), or missing environmental data for one season ($n = 4$). The final dataset comprised 36 sites for which all environmental parameters and biodiversity data were available for both seasons.

2.2 | Environmental Parameters

We estimated discharge at each site by fitting a drainage area–discharge model based on field measurements at selected locations (Burgers et al. 2014). Because it was not feasible to measure depth and flow velocity at every transect, we projected these variables across the network using the fitted model. Drainage area and a digital river network to use for spatial analysis were computed from the Copernicus (European Union's Earth Observation Program) digital elevation model (25 m resolution, EU-digital elevation model v1.1) using the 'WatershedTool' package (Talluto 2020) in R version 4.3.1 (R Core Team 2023).

At each sampling site, we recorded water temperature for 2 to 28 days (mean = 8 days) with a MiniDOT logger (PME, Vista, USA), and filtered a water sample through a sterile 0.2 μm GHP filter and stored it at 4°C pending laboratory analysis. We quantified SO_4^{2-}, Cl^-, and NO_3^- by ion chromatography (Dionex ICS-200; Thermo Scientific Fisher, Waltham, Germany), and Ca^{2+}, Mg^{2+}, K^+, and Na^+ through optical emission spectrometry (ICP-OES, Thermo iCAP600, Thermo Scientific Fisher) following acidification with 32% HCL to pH < 0.2. We excluded ions (Al, B, Fe, Mn, P) with concentrations mostly below detection limit throughout the whole dataset from further analysis. Finally, we reduced dimensionality of z-scaled water chemistry variables to the first two axes of a principal component analysis (PCA) based on data pooled across both seasons.

2.3 | Representation of Space

We used asymmetric eigenvector maps (AEM) computed with the 'AEM' R-package (Blanchet et al. 2007) to represent spatial relationships among sites. AEMs summarize directional fluvial processes and formalize potential spatial autocorrelation among sites; they can be used as predictors for species distributions. AEMs were first proposed by Blanchet et al. (2008, 2011) and were successfully implemented in large scale studies to describe spatial patterns (Pollice et al. 2020; Brasseur et al. 2023; Fuß et al. 2024; Thuile Bistarelli et al. 2024). Briefly, a side-by-edges matrix, which expresses the asymmetric directional connection among sites given by flowing water, is decomposed into a series of latent spatial eigenvectors. Here, we weighted AEMs by water travel time and obtained a set of 11 spatially autocorrelated variables (Dray et al. 2006, Fuß et al. 2024 for details). We use these AEMs as spatial variables

(SV) that represent detectable spatial patterns, which can be interpreted as the result of a spatial process such as dispersal through downstream flow or isolation-driven differentiation among tributaries.

2.4 | Community Data

We sampled the local benthic invertebrate community with two independent multi-habitat samples to be used in two assessment methods. For each, we distributed 20 individual samples to available microhabitats based on estimated areal proportions and sampled these with a quadratic (25 × 25 cm) net with a 500 μm mesh (Hering et al. 2003). We conserved one pooled sample in about 1 L 96% denatured ethanol for metabarcoding ("metabarcoding data") while we fixed the second with formaldehyde (final concentration ~3%) for the standard benthic invertebrate assessment ("standard biotic data"). In the lab, we rinsed the formaldehyde-fixed multi-habitat samples, subsampled to a sixth of the total sample and sorted the benthic invertebrate specimens. If possible, we identified benthic invertebrates to the species or genus level, otherwise we stopped at family level (e.g., Chironomidae), and standardized data to densities (ind. m^{-2}).

From the ethanol-conserved samples, we excluded large particles (> 50 μm), filtered the remaining preservative ethanol (900–950 mL) through sterile 0.45 μm nitrocellulose filters (Nalgene Analytical Test Filter funnel CN, ThermoScientific) and extracted DNA from the filters for DNA metabarcoding following Zizka et al. (2019). For the spring samples, two filters were used for filtering and pooled for extraction because the higher fine particle load impeded the filtering process; this was not necessary for the autumn samples. DNA extraction followed a modified salt-precipitation protocol after proteinase digestion (Sunnucks and Hales 1996; Weiss and Leese 2016). We targeted a 421 bp fragment of the mitochondrial cytochrome oxidase I (COI, barcode region) unit using the BF2/BR2 (Elbrecht and Leese 2017) primers. For a detailed description of our metabarcoding assessment, including bioinformatic analysis, taxonomic annotation and plausibility check, see Brasseur et al. (2023). Briefly, metabarcoding data were first checked with FastQC v.0.11.8 (Andrews 2010) and then processed with the JAMP v0.67 (https://github.com/VascoElbrecht/JAMP) workflow in R (R core Team 2023). DNA metabarcoding reads with 97% sequence similarity were clustered into Molecular Operational Taxonomic Units (MOTUs). For the taxonomic assignment, we used BOLDigger v1.2.2 (Buchner and Leese 2020) implementing searches across the Barcode of Life Data system (BOLD, 18.11.2019) (Ratnasingham and Hebert 2007). Since sampling in the Vjosa River network generates data of a taxonomically underexplored fauna, DNA reference libraries are limited. Therefore, manual curation of the molecular data set was necessary, that is, removal of implausible taxa (e.g., taxa known only from other regions, other continents, or not considered as aquatic invertebrate) or correction to a higher taxonomic level (e.g., species to genus level, yet with a generic species identifier if several species-level reference sequences at comparable levels of sequence similarity were among the 20 best matches of a query sequence). For a species-level assignment, at least 20 query hits > 90% similarity to reference sequences of the same species or a best match with ≥ 98% similarity were required (see Brasseur et al. 2023 for details).

2.5 | Classical Ordination-Based Metacommunity Process Inference

We mapped alpha diversity (taxa richness) at each site for each season and assessment method. Also, we computed the uniqueness of community as the percentage of taxa occurring at a single sampling site. To visualize unconstrained community turnover, we used Bray-Curtis- and Sørensen-based 2D-NMDS for the standard and metabarcoding assessment, respectively, and mapped community turnover as color gradients on maps of the Vjosa River network. To disentangle the effects of environment and spatial structure, we used canonical correspondence analysis (CCA). We reduced multicollinearity of spatial variables (SV) with the 'rdacca.hp' R package (Lai et al. 2022) and retained SVs that explained more than 5% of variance in at least two models (SV: 1, 2, 7, 9, 11). Here, we estimated the importance of single SVs (as adj. r^2) through a hierarchical partitioning across all possible CCA model combinations (2^{11}) for each season and assessment method separately (Table S1). Moreover, we used the four environmental variables (PC 1, PC 2, discharge, water temperature) for the CCA models and partitioned community turnover between environmental and spatial variables. To ensure comparability of CCA models among seasons and assessment methods, we did not select environmental predictor variables in any of the models. To compare ordination results between assessment methods, we used Procrustes analysis on CCA site scores (999 permutations) and mapped procrustes residuals as color gradients to visualize differences between assessment methods. Unconstrained and constrained ordinations were computed based on Hellinger transformed community data and scaled environmental data using the functions 'metaMDS', 'cca' and 'procrustes' from the vegan R package (Oksanen et al. 2020).

2.6 | Association-Aware Metacommunity Process Inference

Recent studies proposed joint species distribution modeling (JSDM) to account for species interactions and thus improve description of metacommunity processes (Warton et al. 2015). In essence, these models aim to explain occupancy patterns of taxa with various predictors (e.g., environmental and spatial variables) and account for among-taxa correlations in the residuals with a residual covariance term that is understood as expressing biotic interactions (or more generally any 'species association'). Notably, it is not possible to separate the residual covariance among species from shared effects of unmeasured spatial or environmental variables. In general, modeling outcomes with high importance of the environment are presumed to indicate environmental filtering, while high importance of space indicates dispersal effects (mass effects, dispersal limitation) as the main determinant of metacommunity structure. A high residual covariance indicates higher importance of species interactions and/or of any shared process that cannot be explicitly linked to the environmental and spatial variables already included in the model. JSDMs allow analysis of the “internal structure of a metacommunity”, that is, a dissection of the different contributions of taxa or sites to the global metacommunity structure (Leibold et al. 2022). More specifically, community turnover is partitioned into environmental (E), spatial (S) and residual covariance (termed association A) components, each indicating possible processes driving (i) the species turnover for each site and (ii) the distribution of each taxon. In detail, we implemented a scaled JSDM-approach by fitting four distinct JSDMs using the ‘sjSDM’ R package v.1.0.6 (Pichler and Hartig 2021) to our dataset: models describing the two standard biotic datasets were fitted to abundance data under a negative binomial distribution and using a probit link while those for the metabarcoding datasets were fitted to presence-absence data using a binomial likelihood and a multivariate probit link. In each JSDM, we implemented the same set of environmental and spatial variables as used in the ordinations as linear terms, where the spatial part was based on the intercept of the environmental one. We included a regularization with a penalty term $\lambda = 0.01$ (penalizing overly complex curves) for biotic interactions and set the weights for lasso or ridge weighting to $\alpha = 0.5$ ($0 =$ pure lasso; $1 =$ pure ridge) for all regression slopes to avoid overfitting or a non-fitting model. The models were run for 150 iterations. Model components environment (E), space (S) and residual covariance among taxa (A) were computed as (absolute) r^2 fractions at global scale, at local scale (i.e., specific for each site and season), and for individual taxa. The site-specific (and the taxon-specific) E, S, A fractions of r^2 add up to the global McFadden r^2. To ease interpretation of results, we distributed shared fractions ($E+S$, $E+A$, $S+A$, $E+S+A$) evenly among involved model components, and computed proportional E, S and A fractions summing to 1 for graphing as pies and in ternary plots.

To closer investigate taxon-specific r^2 fractions with a focus on seasonal changes, we selected 57 taxa from the metabarcoding assessment, which were assigned to species-level, occurring in spring and autumn with $r^2 > 0$. We limited this analysis to metabarcoding data, reasoning that the comparison between standard and metabarcoding assessments may be limited by the fact that there is no conclusive evidence that taxa with identical names correspond to the same evolutionary entity: for example, while an unknown species of the genus *Isoperla* may be recognized as distinct from *Isoperla vjosae* in the metabarcoding dataset, the specimens identified as *Isoperla* in the standard biotic dataset may belong to any of these two taxa or even others. For the selected taxa, we performed two hierarchical cluster analyses (one for each season) based on Canberra dissimilarities among taxa computed from their seasonal E, S and A fractions of r^2 and using the Ward. D2 algorithm for aggregation.

3 | Results

3.1 | Environment

Environmental gradients across the Vjosa River network were similar in spring and autumn (Figure 2). Water chemical gradients represented by PC 1 and PC 2 (Figure S1) can be associated with bedrock and sediments in a geologically diverse basin. PC 1, negatively associated with SO_4^{2-}, Cl^-, NO_3^-, and Ca^{2+} concentrations, decreases from the mountainous region in the Aoos, Sarantaporos, and Luftinja towards downstream (Tables S2 and S3). In contrast, PC 2, negatively associated with Mg^{2+}, K^+, and Na^+ concentrations, is highest in the calcareous tributaries Voidomatis, Drinos, and Shushica and low in the Luftinja and Langarica (Tables S2 and S3). Except for a higher variation of PC1 for autumn, water chemical gradients were similar between seasons (PC 1 Pearson's $R = 0.91$, $p < 0.001$; PC 2 $R = 0.94$, $p < 0.001$). Water temperature increased from the mountain regions to the lowlands, where highest values are found in the smaller tributaries Luftinja and Shushica. Water temperature was generally higher in autumn, but gradients strongly agreed between seasons ($R = 0.9$, $p < 0.001$). Discharge increased with stream order to a maximum of about 75 $m^3\ s^{-1}$ and 31 $m^3\ s^{-1}$ at the most downstream sampling site in spring and autumn ($R = 0.99$, $p < 0.001$), respectively.

3.2 | Space

To account for the asymmetric spatial structure of the Vjosa River network we implemented the most important (hierarchical partitioning, Table S1) spatial variables (SV 1, 2, 7, 9, 11) derived from AEM in both the classical ordination data analysis and the JSDM analysis. Here, SV 1 represents a large-scale up- to downstream gradient, SV 2 captures the differentiation of midstream Vjosa locations (Figure 3: SV 2 blue points) vs. the tributary Drinos (SV 2 red points), SV 7 clearly separates Voidomatis (SV 7 red point) and the lower Aoos section (SV 7 blue points), SV 9 mainly separates the lower Sarantaporos (SV 9 red point) from the Aoos, and SV 11 captures spatial variation in the upper Sarantaporos (Figure 3).

3.3 | Unconstrained Analysis of Community Turnover

Across the 36 sites in spring, we recorded 376 and 181 taxa in metabarcoding and standard biotic data, respectively, while in autumn we recorded 276 and 136 taxa, respectively. At individual sites in spring, metabarcoding and standard biotic data comprise on average 61 ± 19 (mean ± SD) and 36 ± 13 taxa, respectively. In autumn, richness was lower with an observed site-wise average of 52 ± 20 taxa in the metabarcoding data and 25 ± 11 taxa in the standard biotic data. Generally, higher taxon richness was observed in most low order streams. A site with particularly high richness observed with both datasets and in both seasons was the lower Aoos section after the confluence with the Voidomatis. Metabarcoding and standard data differed strongly in some mainstem sites in autumn (with metabarcoding data indicating greater richness) and in the Luftinja and Dishnica tributaries in spring, again with a much greater richness in metabarcoding data while the standard biotic data suggest a particularly poor community. Spatial community turnover, that is, beta diversity, was assessed through unique taxa and dissimilarity analysis: with the standard and the metabarcoding assessment we detected 25 and 52 unique taxa in spring and 2 and 55 unique taxa in autumn, respectively. Plotting site scores of the first and second axis of a dissimilarity-based 2-dimensional NMDS at their geographical sites, we observed a high turnover from the headwaters (of

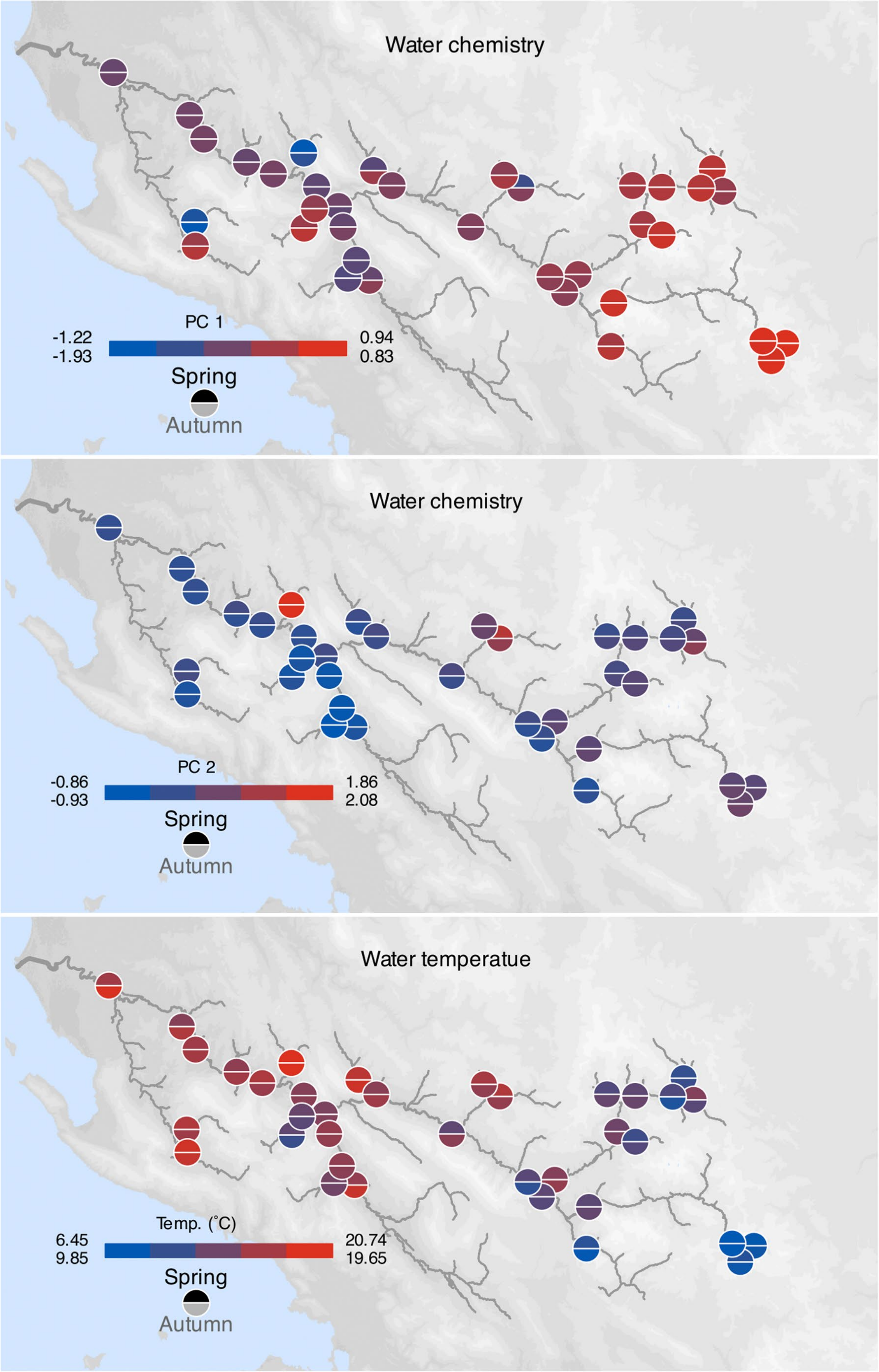

FIGURE 2 | Legend on next page.

FIGURE 2 | Environmental variables in spring (upper half circle) and autumn (lower half circle) 2018 in the Vjosa River network. Upper panels show water chemistry gradients summarized by the first (PC 1) and second (PC 2) axis of PCA, respectively. The lower panel shows water temperature in the network. Gradients go from blue (low) to red (high) and are defined for each variable and season separately.

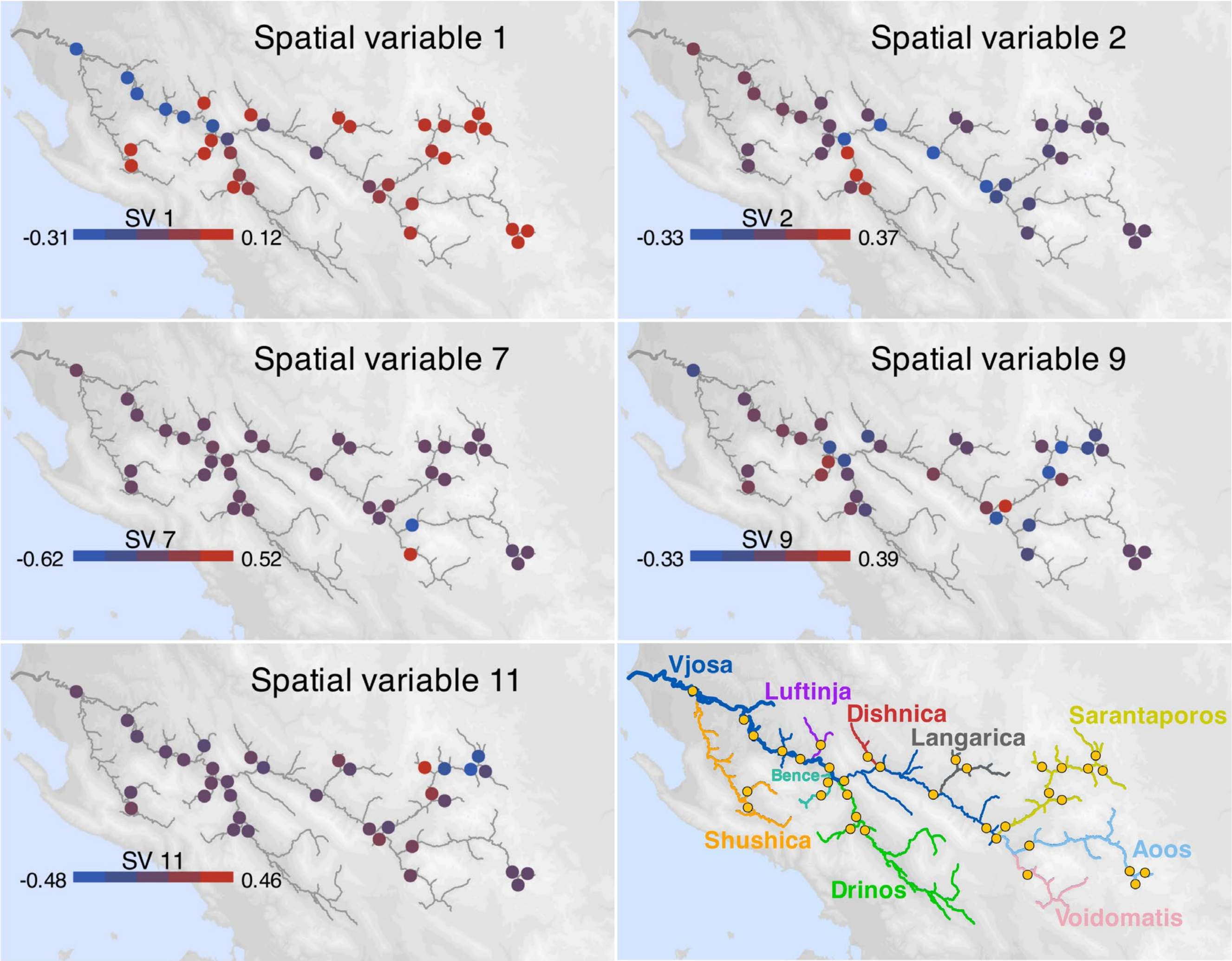

FIGURE 3 | Selected spatial variables (SV) associated with large-scale (SV 1, 2) and small-scale (SV 7, 9, 11) turnover of the benthic community in the Vjosa River network. SVs are based on Asymmetric Eigenvector maps. Gradients go from blue (low) to red (high) and are redefined for each SV.

the Sarantaporos, Aoos and Voidomatis) towards the downstream section of the main stem in both seasons and with both assessment methods. In addition, in spring, we observed notable turnover from the southern tributaries towards the main stem and the northern tributaries (Figure 4).

3.4 | Spatially and Environmentally Constrained Community Turnover

For the metabarcoding data, community turnover across the river network was associated with the same water chemistry variables (PC1 and PC2), water temperature, discharge, and the spatial variable SV1 in both seasons. These variables slightly changed their contributions to CCA axes between seasons, while other (mostly spatial) variables remained less important. Despite changes in loading between seasons, the differences in water chemistry, water temperature, discharge, and relative position in the stream network as captured by SV1 clearly separated headwater and main channel communities in the ordination.

Applying CCA to the standard biotic data identified other variables as important for explaining community variation in the two seasons (Figure 5): in spring, we found PC1 and PC2, water temperature, discharge, and the spatial variable SV1 to be important, yet also other spatial variables were contributing to the definition of the ordination space. The importance of spatial variables was even more pronounced in the ordination of the autumn standard biotic data. Also, in this dataset, main channel sites appeared to be more similar to each other and were more homogeneous in their environmental conditions, while variation of communities as well as of environmental and

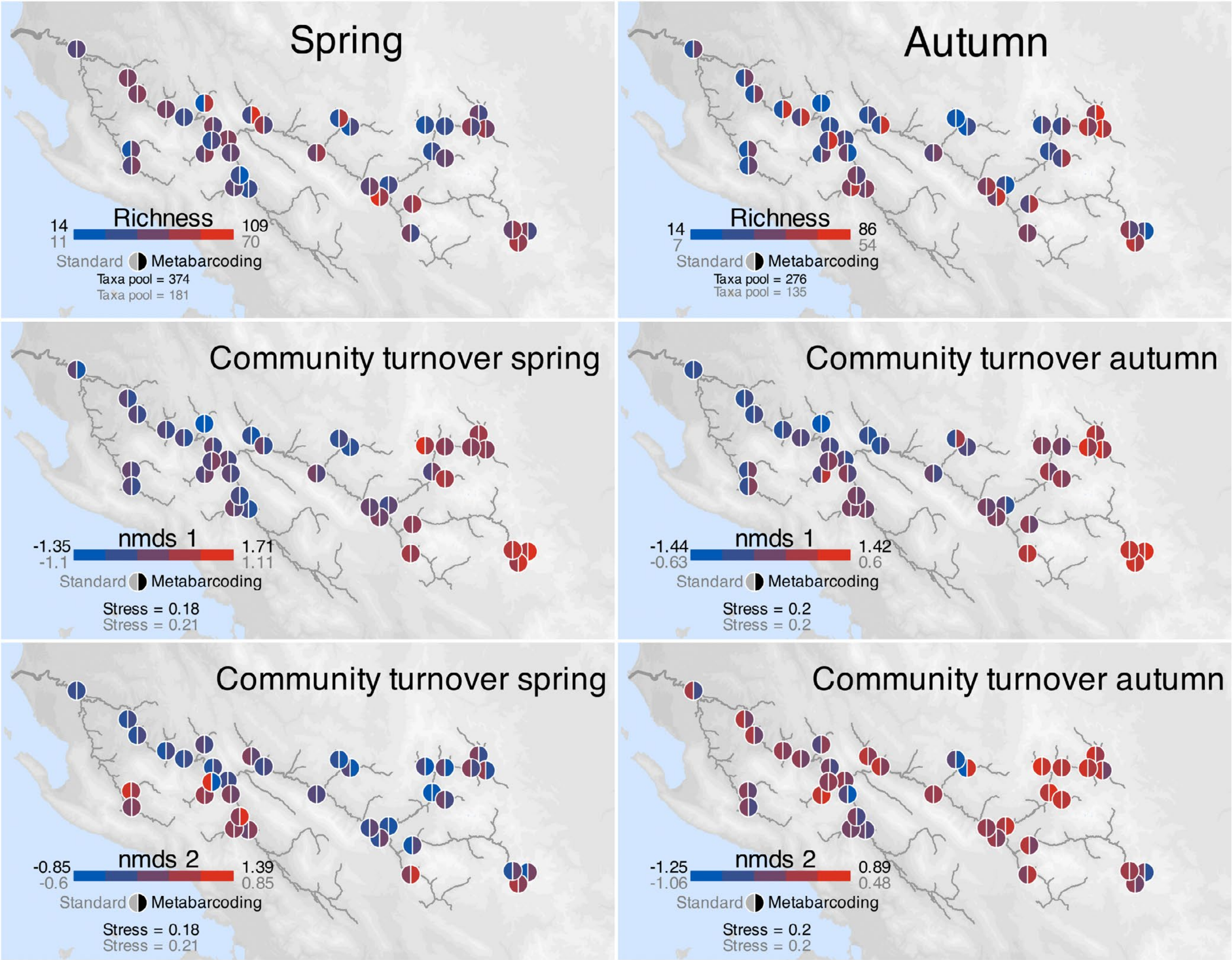

FIGURE 4 | Gradients of alpha and beta-diversity in the Vjosa River network. Uppermost panels show taxa richness in spring (left) and autumn (right). Middle and lower panels show the site scores on the first and second dimensions of NMDS ordinations, respectively. Sampling sites are shown as 2 half circles: the left half circle indicates assessments based on standard biotic data while the right half circle shows the metabarcoding data. Gradients go from blue (low) to red (high), their ranges are defined for each assessment and season separately.

spatial variables was greater in the tributaries and headwaters (Figure 5, Figure S1).

Summarized for both assessment methods, variation partitioning based on the CCAs indicated that environmental variables explained 5%–9% and spatial variables 1%–11% of the community turnover. A relatively large fraction of 3%–9% was explained by combined environmental and spatial variables, indicating a spatial structure in environmental variables. However, CCA resulted in large residual variation, as 78%–88% of the community turnover could not be explained by predictor variables. Overall, space appears to be marginally more important in explaining community turnover in autumn while the environment is slightly more important in spring (Table 1).

To focus on commonalities and simultaneously compare results between assessment methods, we rotated CCA spaces inferred from standard biotic data to match those inferred from metabarcoding data by Procrustes analysis. We observed significant ($p = 0.001$) correlations for both seasons; largely concordant patterns across the river network between assessment methods were also visible by quite similar spatial configurations in ordination space (Figure S2), especially in spring data. The disagreement between ordinations of the two assessment methods was generally higher for autumn data (higher residual range). The greatest divergence between assessment methods was observed in the headwater sites in spring (in the Luftinja, Sarantaporos and Aoos/Arkoudoremas in particular); this pattern persisted in autumn with additional divergence between standard and metabarcoding datasets in the centrally located tributary Bence (Figure 6).

3.5 | Association-Aware Metacommunity Patterns

The JSDM models indicated a high variability in the effects of environmental and spatial variables and in the residual covariance among taxa, between seasons and between assessment methods. For metabarcoding data, we calculated a global McFadden r^2 of 0.61 for spring and 0.54 for autumn. In comparison, for the standard biotic data we had a lower McFadden r^2 of 0.25 and 0.24 for spring and autumn, respectively. In spring,

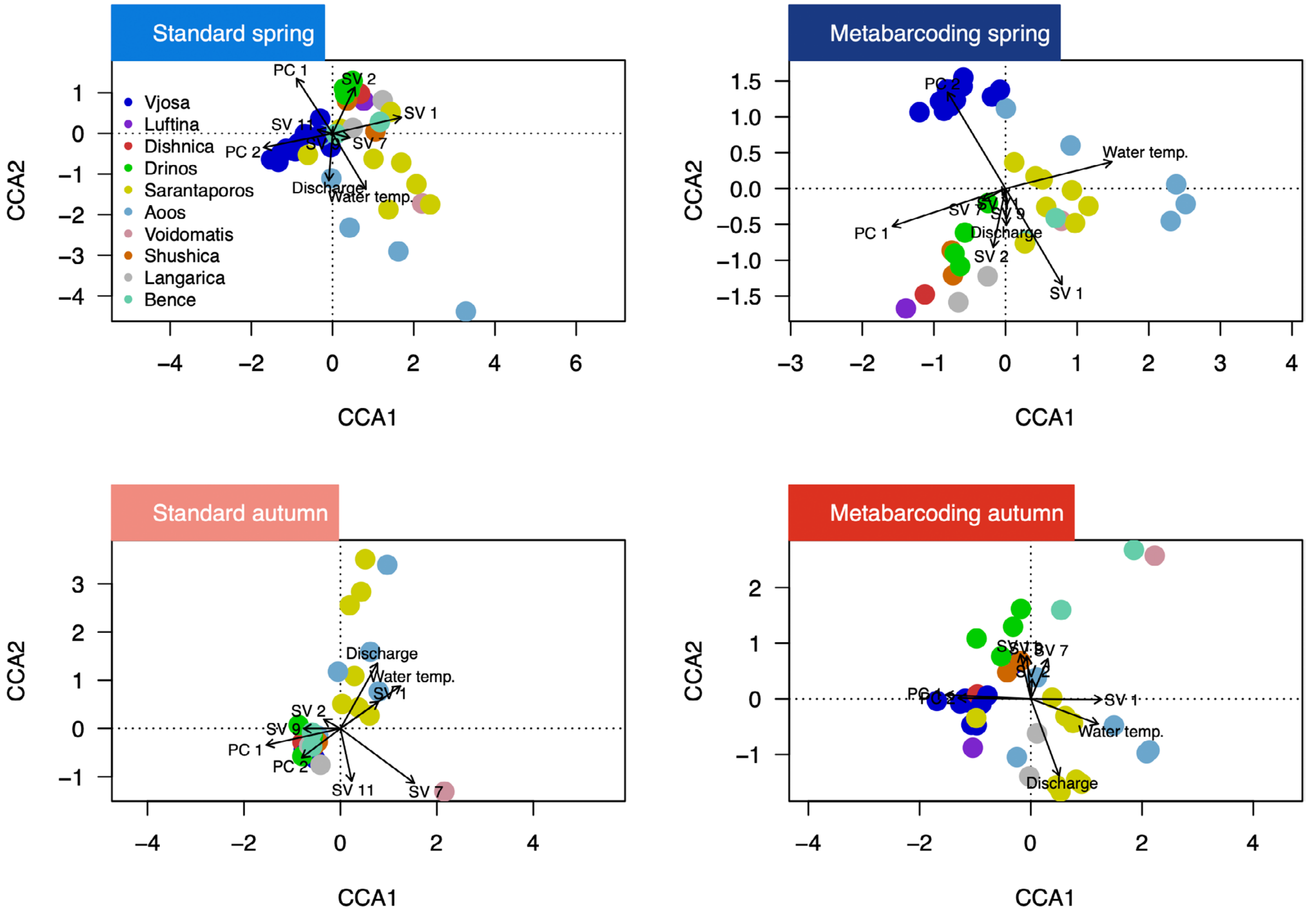

FIGURE 5 | Environmentally and spatially constrained community structure in the Vjosa River network assessed with a standard (left) and a metabarcoding (right) assessment in two seasons in 2018. In spring after high flow (blue, upper) and in autumn after summer low flow (red, lower). Black arrows indicate the loadings of environmental and spatial predictors.

TABLE 1 | Results from CCA permutation tests for each season and assessment method describing effects of environmental and spatial constraints on the community data.

Assessment	Season	Chi^2, Df=9	*F*	*p*	Res. chi^2, Df=26	*E* (%)	*S* (%)	*E* & *S* (%)	Res.
Metabarcoding	Spring	1.7	1.6	***	3.2	9	1	3	87
Metabarcoding	Autumn	1.4	1.5	***	2.7	5	3	4	88
Standard	Spring	1.5	2	***	2.2	5	6	9	79
Standard	Autumn	1.9	2	n.s.	2.6	6	11	5	78

Note: We used variation partitioning to compute the (global) fractions explained by environment alone (E), by space alone (S), and by space & environment combined (E & S). *** indicate significant effects.

most of the community turnover in metabarcoding data was explained by residual covariance of taxa ($A=0.28$), followed by environment ($E=0.21$) and space ($S=0.12$). Community turnover in metabarcoding data in autumn was again largely explained by the residual covariance among taxa ($A=0.29$), and in similar amounts by environment ($E=0.12$) and space ($S=0.12$).

The higher share of variance explained by residual covariance of taxa at downstream located sites is contrasted by environment and space describing more variance in the headwaters in both seasons. JSDM results based on standard biotic data from spring indicated a higher residual covariance of taxa ($A=0.09$) in the mainstem, with environment ($E=0.08$) and space ($S=0.08$) explaining more variance in the headwaters. In autumn standard biotic data, we observed a higher effect of environment ($E=0.15$) also along the mainstem, space ($S=0.02$) explaining variation in the headwaters as well as some main stem sections, and an overall lower importance of residual covariance of taxa ($A=0.07$) (Figure 7; for a definition of headwaters vs. mainstem sites, see Figure 1).

Closer inspection of variance partitioning results at the scale of local sites revealed seasonal patterns: From spring to autumn, we observed a general increase of the relative importance (as

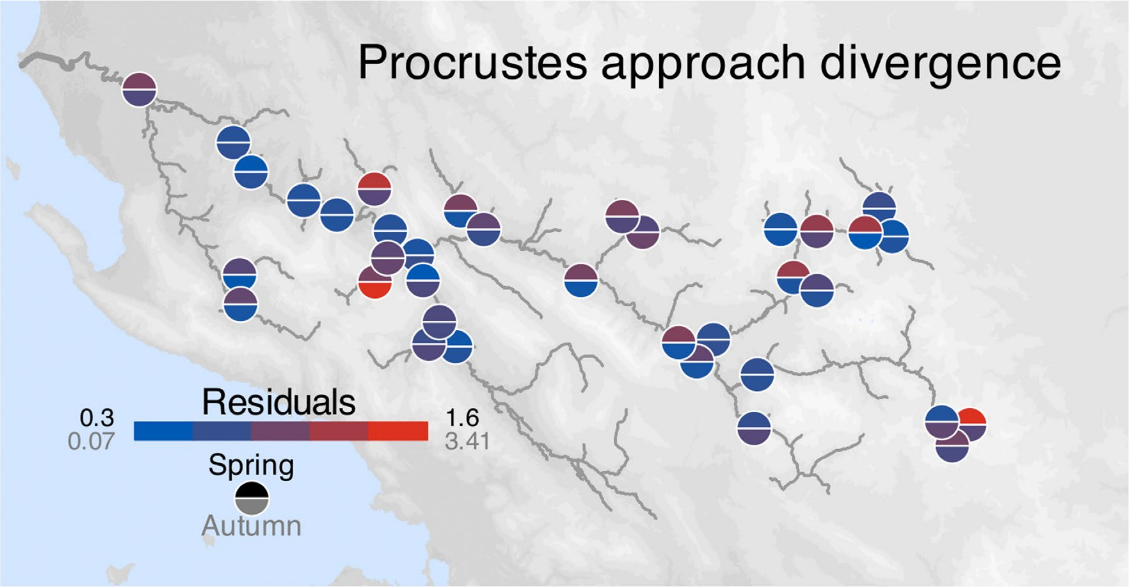

FIGURE 6 | Procrustes comparison between CCAs inferred from metabarcoding data and from standard data in spring (upper half circle) and autumn (lower half circle) 2018 in the Vjosa River network. Color gradient indicates the min (blue) to max (red) range of the residuals, CCA ordinations were significantly ($p = 0.001$) correlated between assessments in both seasons. Lower residuals indicate greater agreement between methods.

proportional r^2 fraction) of the residual covariance among taxa and, to a lesser degree, of the environment but a decrease in the importance of space. This seasonal shift was more obvious in metabarcoding data (Figure 8).

We also found that taxa differed strongly in their responses to environment, to space and in their residual covariance with other taxa (Figure 8 lower panels). While some taxa were strongly controlled by local environmental conditions, others appeared to be mostly controlled by spatial variables or mostly driven by residual covariance with others (especially with the metabarcoding assessment). Generally, the benthic invertebrate community appears to be controlled by a complex interplay of environment, space and residual covariance among taxa in both seasons and in both biotic datasets. Also, for the standard biotic data, the model suggests for many taxa that the high importance of environment and space in spring shifts to high importance of residual covariance among taxa in autumn. This, however, is not as clear with data from the metabarcoding assessment (Figure 9).

Investigating r^2 fractions for selected taxa revealed that the relative importance of spatial or environmental variables or the residual covariance among taxa can change between seasons. The cluster analyses resulted in 3 distinct clusters for spring and autumn, respectively, which were easily characterized by the dominant r^2 fraction: Cluster 1, 2 and 3 contain species with high r^2 fractions attributed to residual covariance among taxa, environmental or spatial variables, respectively. Closer inspection allowed finer discrimination of further clustering; notably a few species apparently experienced no spatial controls. Moreover, some species were recovered in the same cluster in spring and autumn, while others changed from one cluster to another, that is, they showed a shift of controlling variables between seasons (Figure 3). Differences between taxa from the same genus (e.g., *Hydropsyche* sp., *Leuctra* sp., *Perla* sp., *Rhithrogena* sp., *Simulium* sp.) underline the importance of working at species-level (Figure 10).

4 | Discussion

We found clear community turnover patterns from headwaters and tributaries towards the mainstem of the Vjosa River network, prominently driven by spatial constraints, environmental heterogeneity, and also associations among taxa. These patterns were not completely in line with our initial hypotheses about the shifting importance of environmental and spatial regulators along the river network. Moreover, our attempts to use classical ordination-based approaches to infer the relative importance of space, environment, and their combination were largely inconclusive, with high model residuals. In addition, we observed substantial differences among the patterns inferred by jSDM based on standard and metabarcoding community data.

We hypothesized that headwater tributaries should be structured mainly by spatial and environmental constraints, in line with earlier results and evolutionary theory (Heino et al. 2015; Altermatt 2013; Fuß et al. 2025). However, our results point to an at least similarly important influence of associations among taxa. Also, this pattern is stable across seasons and methods. Possibly, priority effects among distant headwater tributaries shape assembly processes more strongly than space or environment alone, but also associations based on trophic co-variation could be important—e.g., of benthic invertebrate shredders that rely on the greater supply of allochthonous organic matter in headwater tributaries (Zou and Rudolf 2023; Talluto et al. 2024). While our hypothetical framework suggested a higher-than-found relevance of associations among taxa in headwater tributaries, it accounted for association-driven community assembly in mid-stream reaches and larger

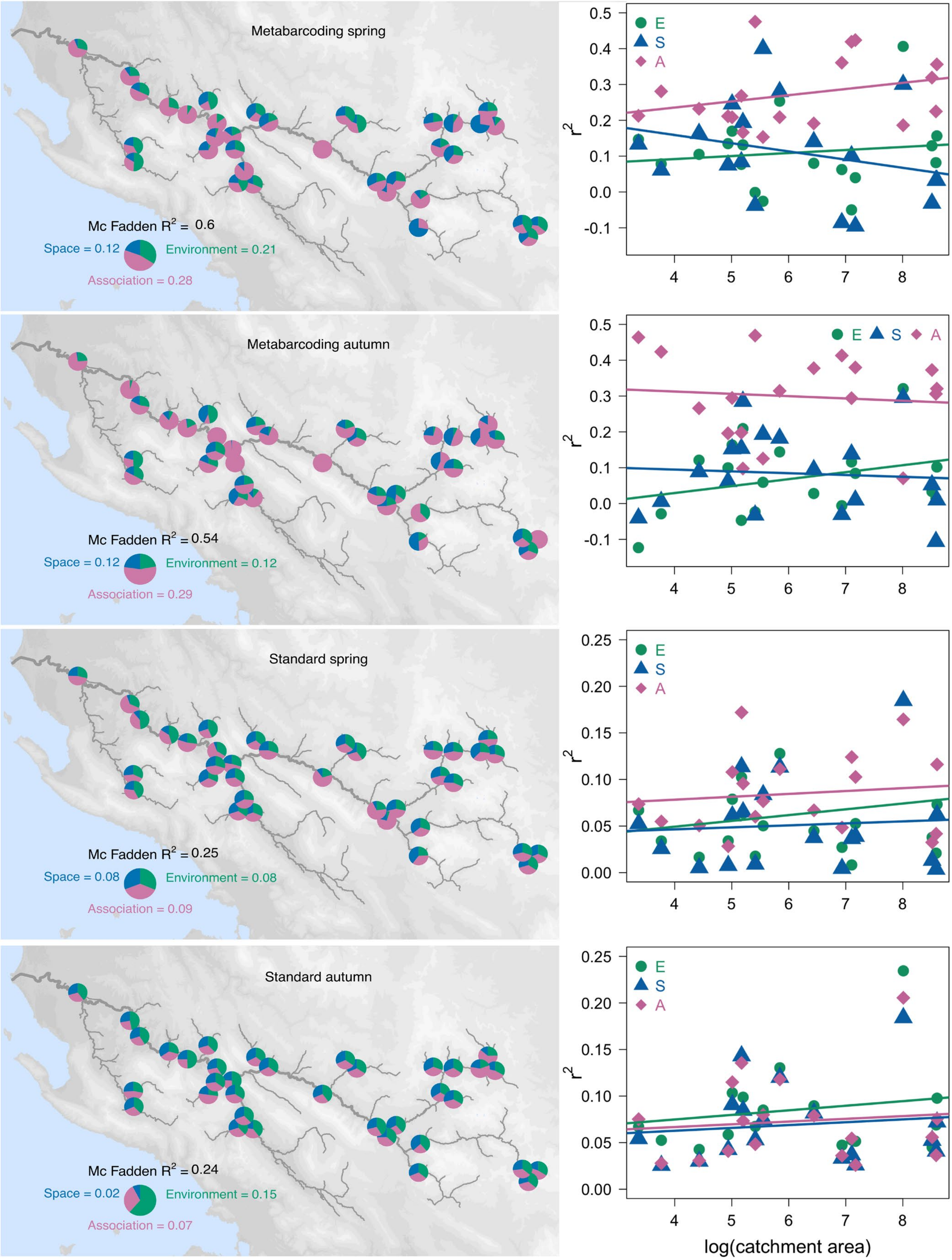

FIGURE 7 | Legend on next page.

FIGURE 7 | Pie charts in the Vjosa River network show the proportional r^2 fractions for environment (*E*, green), space (*S*, blue) and residual covariance among taxa (*A*, pink) that describe the community composition at each sampling site for the metabarcoding and the standard biotic data during spring and autumn 2018. The lower left corner shows the global r^2 (McFadden) and proportional global r^2 fractions. Side panels show how local r^2 fractions change in relation to log catchment size.

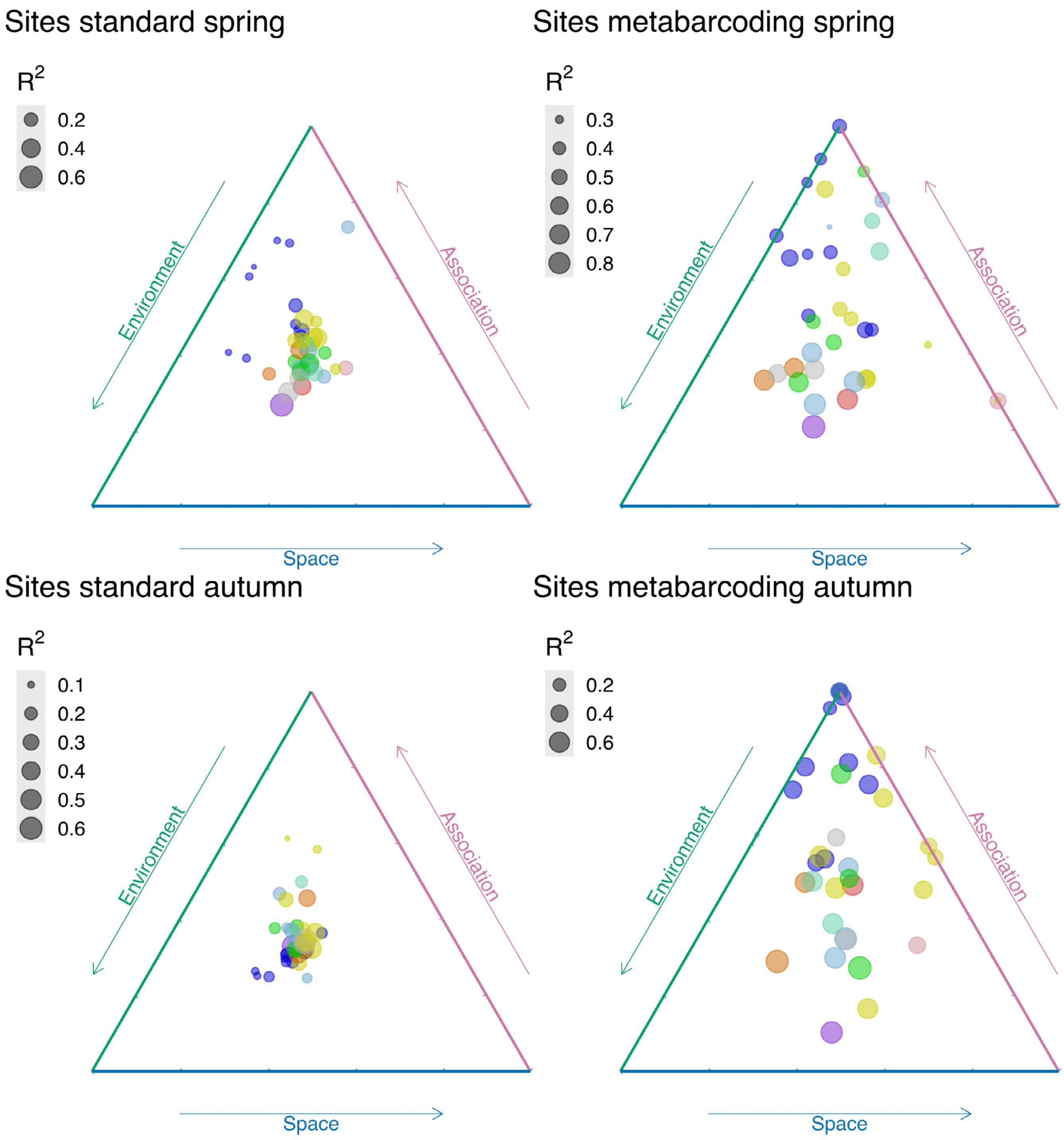

FIGURE 8 | Each point represents a sampling site in the Vjosa River network and is positioned in relation to proportional r^2 fractions *E*, *S* and *A*, that is, how much the community composition is explained by environment (*E*, green), space (*S*, blue) and the residual covariance among taxa (*A*, pink). High values for *E* (lower left corner) are interpreted as environmental filtering, high values for *S* (lower right corner) indicate dispersal-mediated mechanisms, high values for *A* indicate potential biotic interaction. Data originate from standard (left) and metabarcoding (right) assessment in spring and autumn, respectively. Color used for sites indicate tributary identity while circle size indicates r^2 for each site and taxon, respectively.

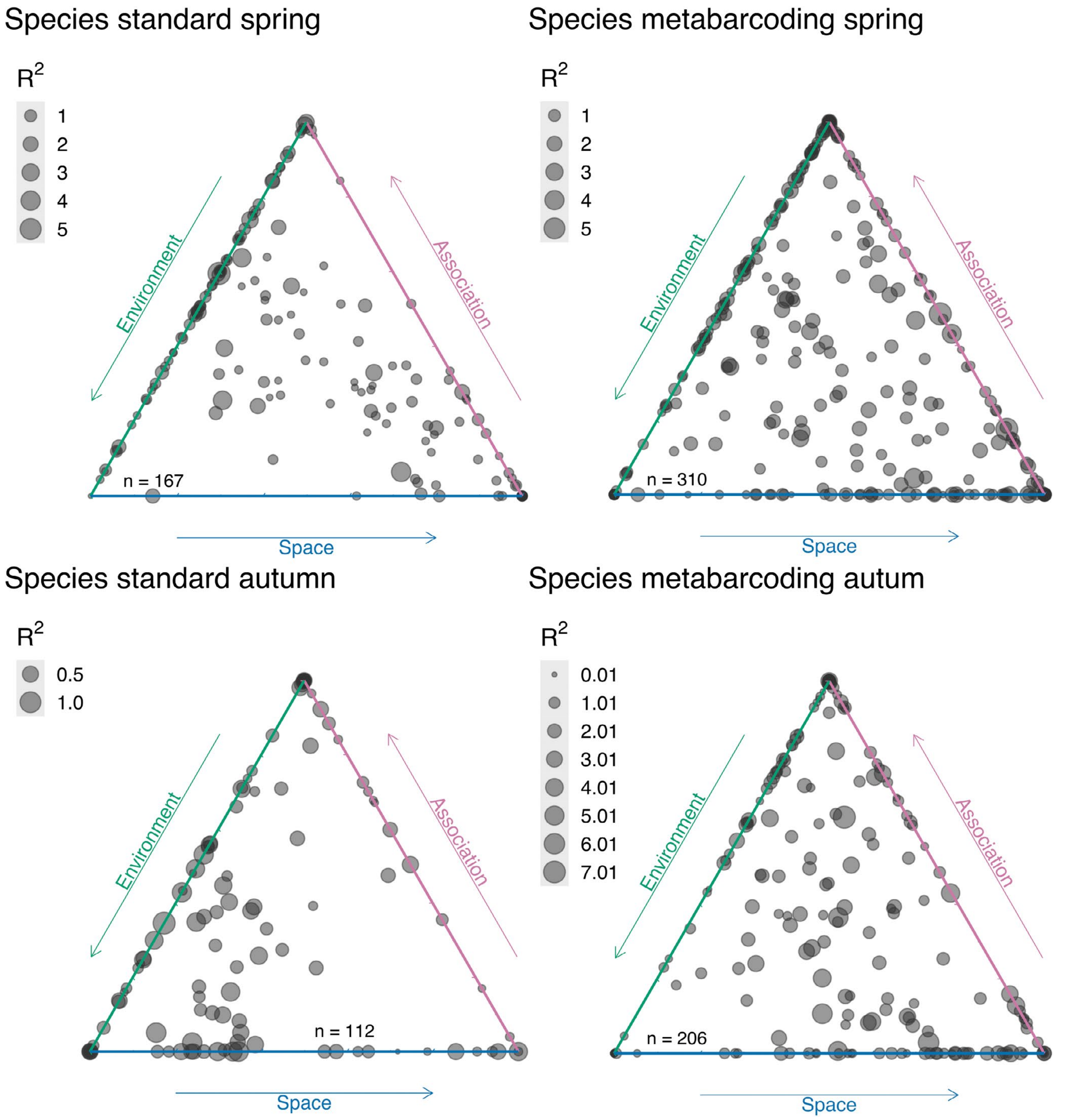

FIGURE 9 | Each point represents a taxon in the Vjosa River network, is positioned in relation to proportional r^2 fractions, and indicates how much a taxon is explained by environment (E, green), space (S, blue) and the residual covariance among taxa (A, pink). Data originate from standard (left) and metabarcoding (right) assessments in spring and autumn, respectively. Circle size indicates r^2 for each site and taxon, respectively.

mainstem tributaries. Indeed, we found that environment and associations among taxa were the most prominent controls of assembly processes in upper mainstem sites and mainstem tributaries, with spatial constraints of lesser importance, while metabarcoding data paint a different image. Apparently, associations among taxa become more important than spatial and environmental constraints, the larger the system becomes—and this also pertains to the mainstem sites, for which we predicted space and associations as key controls of assembly processes. Indeed, we found that the importance of space and environment decreased towards the mainstem and the downstream sites while associations among taxa apparently explain the majority of variation among communities. This is somewhat counter-intuitive in light of previous results, that—as our hypotheses—suggest stronger spatial effects acting on communities of the environmentally homogeneous lower mainstem sites (Tonkin et al. 2018; Stephan et al. 2021; Fuß et al. 2025). Overall, our results are difficult to set into context based on literature, also because our study is one of the first to use jSDMs in context of metacommunity analysis.

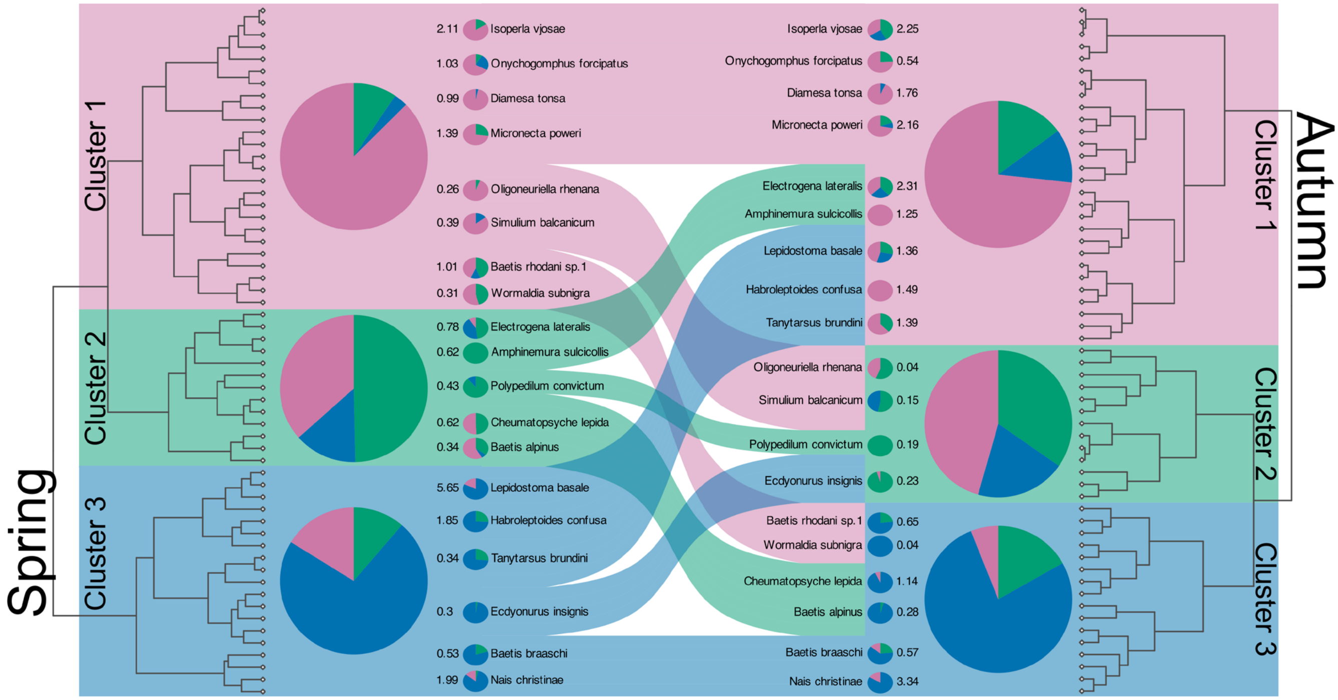

FIGURE 10 | Cluster analyses based on *E*, *S* and *A* r^2 fractions of 57 selected taxa from the metabarcoding dataset resulted in three characteristic clusters for spring (left) and autumn (right); each cluster contained species dominated by environmental (*E*, green) or spatial (*S*, blue) variables or showing high residual covariance among taxa (*A*, pink). Pie charts show proportional r^2 fractions attributable to *E*, *S* and *A* for a subset of representative species (small pies) or on average for each cluster (large pies). The central flow chart (Sankey diagram) displays the seasonal shift of many species among clusters.

Associations among taxa, that is, the residual covariance among taxa which is assumed to represent "biotic interactions", are difficult to explain. In heterogeneous and highly productive habitats this measure may reflect the multitude of interactions in a food web (Ward et al. 2023; Scholl et al. 2023). Along this line of reasoning, the high degree of interactions would allow the consumer community to adapt to regular disturbance (e.g., by high fine sediment loads and river channel translocation) and confer stability in a highly variable and dynamic ecosystem (McCann and Rooney 2009, Jacquet et al. 2016, 2022). Also, residual covariance might relate to patch-scale colonization success of an individual taxon depending on the prior community, possibly modulated by the environmental or spatial context (Zou and Rudolf 2023). The challenge here is that residual covariance among taxa may result from a range of mechanisms. Residual covariance or "biotic interactions" are potentially more sensibly interpreted as a latent variable that describes a shared effect of unmeasured habitat or community properties—also because a dataset on benthic invertebrate fauna alone cannot depict all relevant associations within a natural community.

In addition to these methodological constraints, our seasonal comparisons imply that reality is highly complex. The varying importance of metacommunity processes between seasons suggests that the mechanics of community assembly change regularly. Seasonal shifts could be related to phenology, that is, shifts within the community related to life cycle and ontogenetic development. This interpretation is probably best illustrated using an example: The mayfly *Oligoneuriella rhenana* has a synchronized life cycle and is present throughout the catchment. In spring, this species is highly abundant as large-bodied nymphs, ready to hatch and procreate; in autumn, their efforts are reflected in a filial generation of very small early-stage larvae (Bauernfeind and Humpesch 2001). This life cycle translates to seasonal differences in susceptibility to and capacity to engage in metacommunity processes—effectively changing importance among environmental, spatial and association-based determinants of metapopulation dynamics for this species.

Benthic invertebrate fauna comprises a great number of species that have similar—albeit differently timed—life cycles. Also, there is a range of traits related to body size of benthic invertebrates that change with larval development and thus influence their ecological niche, dispersal potential and the way they interact with other biota. In a generalizable framework, spatial variability in oviposition sites and adult dispersal should result in patchy distribution of young instars (Dwyer et al. 2024)—these should additionally undergo much stronger selection, perhaps even across various obligatory microhabitats (Martini and Waringer 2021), and grow into a local interaction network at a specific location. Here, environmental factors and associations among taxa might become more important for older developmental stages whereas the distribution and abundance of younger instars could be more importantly regulated by spatial factors. The above exemplified *O. rhenana* provides mixed evidence for this framework: while a higher importance of the residual covariance with other taxa in spring agrees with developed nymphs "settled" within spring communities, environmental control increased in importance in autumn besides associations with other taxa, but spatial constraints were never found as important for this species. Other taxa may serve as better examples—*Cheumatopsyche lepida*, *Baetis alpinus* or *B.*

rhodani—as here the importance of spatial constraints increases in autumn, even if the *Baetis* species potentially are polyvoltine (Humpesch 1979; Erba et al. 2003; Sand and Brittain 2009). Anyhow, the generalized framework we propose acknowledges that ontogenesis and life cycle dynamics must be expected to influence how metacommunity processes determine local occurrence and abundance of a given taxon at a given time in a given community.

On top of phenological shifts, temporal variation in environmental conditions may enforce spatial constraints only in one season but environmental constraints in another. The Vjosa River shows strong seasonal hydrological fluctuations typical of alpine gravel-shifting rivers: high discharge in spring, low discharge in summer with relatively strong precipitation-induced variation, moderate discharge in autumn, and low discharge again in winter. Potentially, this enforces spatial controls of metacommunity assembly in spring due to greater drift propensities (Kennedy et al. 2014), but could also impose environmental constraints through riverbed disturbance and sediment transport (Brittain and Eikeland 1988). Vice versa, relatively stable low flow conditions during summer or winter may allow for stronger local environmental sorting (Tonkin et al. 2018) and establishment of local associations among taxa.

There are, however, important limitations of our data and results. Foremost, the lacking congruence of results from standard and molecular data impedes generalization. While it is common to recover different taxa or numbers of taxa in metabarcoding data compared to standard approaches (Brasseur et al. 2023), our results suggest that the two data types return deviating ecological signals. Similar observations have been made in context with aquatic ecosystem assessment, but have so far been regarded a mostly technical challenge (Jones et al. 2025; Macher et al. 2025; Šamulková et al. 2025)—even if taxon-specific detection probability varies among and within taxa as well as between metabarcoding and standard morphological approaches with ontogenetic stage and size (Emmons et al. 2023; Gold et al. 2023). Yet, although the typically greater taxonomic resolution of metabarcoding is seen to outweigh the lack of abundance data (Emilson et al. 2017; Remmel et al. 2024; Serrana et al. 2019), it is conceivable that this becomes a disadvantage when investigating controls of metacommunity assembly: for instance, it might become difficult to estimate the degree of association among taxa if only their presence is known but not their abundance which would reflect the local role of a taxon (Avolio et al. 2019). Moreover, taxonomic resolution is a critical factor in analyzing metacommunity processes. Ideally, ecological analyses work at the level of species as the ecologically relevant entities (Göthe et al. 2013; Heino 2014). However, as the number of taxa in a dataset increases, the probability of missing biotic or abiotic parameters that control local presence and abundance increases—thus, it is possible that the proportion of unaccounted-for co-variance (i.e., "associations") among taxa increases in turn.

Setting our results in context with existing literature is additionally challenging, as our study is one of the first to report empirical outcomes of jSDM analyses on metacommunity processes in streams. Beach meiofauna was previously found to show strong associations among locally co-occurring taxa that decreased with increasing environmental distinctness among locations (Pichler et al. 2025), but this seems a trite observation. Conversely, associations among taxa were found to be a source of annual variation in site occupancy in freshwater snails (Dubart et al. 2022). Seasonal differences in controls of metacommunity assembly, however, are rarely considered.

5 | Conclusion

Overall, our results suggest an inherent instability of metacommunity processes and showcase the difficulties associated with their assessment. Obviously, different types of data on the same communities will produce substantially differing and sometimes contrasting outcomes. The comparative analysis of the "internal structure" of the Vjosa River's benthic invertebrate community across two seasons in fact invites to understand dispersal and environmental filtering as what they really are: processes rather than fixed results as suggested by the majority of studies relying on a global variation partitioning of data from a single campaign only. Snapshot studies inevitably lead to the presumption of consistent drivers and stable equilibrium states, and so fail to capture how community assembly, metacommunity processes and natural seasonal dynamics of biota and habitat interact across various time scales. Our first assessment of seasonality in metacommunity processes in the largely free-flowing Vjosa River draws the picture of a naturally pulsing fluvial metacommunity in a hydrologically dynamic riverscape.

However, our results also suggest that river fragmentation (e.g., dams or excessive water abstraction) may have dramatic consequences at larger spatial scales by curbing metacommunity processes. Any barrier to the four riverine dimensions simultaneously trammels environmental filtering, dispersal, and species interactions. While effects of fragmentation on the dynamics of metacommunity processes should be future research subjects, our metacommunity-aware biodiversity assessment of the Vjosa River already offers an opportunity to set reference points for river conservation and targets for renaturation efforts. Disclosing such reference points will be crucial to halt the freshwater biodiversity decline. It is evident that primary conservation targets must include an immediately effective moratorium on modification of near-natural riverscapes—of the Vjosa River and beyond—as well as a reinstatement of natural riverine processes and connectivity at scales as large as possible in already impacted river networks.

Author Contributions

Jan Martini: data curation (lead), formal analysis (lead), investigation (lead), methodology (equal), project administration (equal), resources (equal), software (equal), validation (equal), visualization (lead), writing – original draft (lead), writing – review and editing (lead). **Thomas Fuß:** conceptualization (equal), data curation (supporting), formal analysis (supporting), investigation (supporting), validation (equal), visualization (supporting), writing – original draft (supporting), writing – review and editing (equal). **Marie V. Brasseur:** formal analysis (equal), investigation (equal), methodology (equal), validation (equal), visualization (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Rebecca Oester:** data curation (equal), formal analysis (equal), investigation (equal), visualization (supporting), writing – original draft (equal), writing – review and editing (supporting). **Lauren Talluto:** conceptualization (supporting), data curation (equal), formal analysis (equal), investigation (equal), software (equal), validation (supporting), visualization (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Franziska Walther:** conceptualization (supporting), data curation (equal), formal analysis (equal), funding acquisition (supporting), investigation (equal), project administration (supporting), validation (supporting), visualization (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Olivia Wilfling:** investigation (equal), visualization (supporting), writing – original draft (supporting). **Vera M. A. Zizka:** data curation (supporting), formal analysis (supporting), investigation (equal), methodology (equal), validation (supporting), visualization (supporting), writing – original draft (supporting), writing – review and editing (supporting). **Gabriel Singer:** conceptualization (lead), formal analysis (supporting), funding acquisition (lead), investigation (equal), methodology (equal), project administration (lead), resources (lead), supervision (equal), validation (supporting), visualization (supporting), writing – original draft (supporting), writing – review and editing (equal). **Simon Vitecek:** conceptualization (equal), data curation (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), methodology (equal), project administration (equal), resources (equal), supervision (lead), validation (equal), visualization (equal), writing – original draft (equal), writing – review and editing (equal).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and code are available for review under the following link https://fileshare.uibk.ac.at/d/b87b0e840d9e497eb04d/ and will be made fully accessible upon acceptance of the manuscript with the Zenodo repository: Martini, J., Fuß, T., Brasseur, M. V., Oester, R., Talluto, L., Walther, F. E., Wilfling, O., Zizka, V. M. A., Singer, G., and Vitecek, S. (2025). Dynamics of a free-flowing benthic invertebrate metacommunity [Data set]. Zenodo. https://doi.org/10.5281/zenodo.16021297.

References

Albert, J. S., G. Destouni, S. M. Duke-Sylvester, et al. 2021. “Scientists' Warning to Humanity on the Freshwater Biodiversity Crisis.” *Ambio* 50, no. 1: 85–94. https://doi.org/10.1007/s13280-020-01318-8.

Altermatt, F. 2013. “Diversity in Riverine Metacommunities: A Network Perspective.” *Aquatic Ecology* 47, no. 3: 365–377. https://doi.org/10.1007/s10452-013-9450-3.

Andrews, S. 2010. “FastQC: A Quality Control Tool for High Throughput Sequence Data. (Version FastQC-v.0.11.8.).” http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Avolio, M. L., E. J. Forrestel, C. C. Chang, K. J. La Pierre, K. T. Burghardt, and M. D. Smith. 2019. “Demystifying Dominant Species.” *New Phytologist* 223, no. 3: 1106–1126. https://doi.org/10.1111/nph.15789.

Bauernfeind, E., and U. H. Humpesch. 2001. *Die Eintagsfliegen Zentraleuropas (Insecta: Ephemeroptera): Bestimmung und Ökologie*, 239. Verlag Des Naturhistorischen Museums.

Birk, S., W. Bonne, A. Borja, et al. 2012. “Three Hundred Ways to Assess Europe's Surface Waters: An Almost Complete Overview of Biological Methods to Implement the Water Framework Directive.” *Ecological Indicators* 18: 31–41. https://doi.org/10.1016/j.ecolind.2011.10.009.

Blanchet, F. G., P. Legendre, and D. Borcard. 2008. “Modelling Directional Spatial Processes in Ecological Data.” *Ecological Modelling* 215, no. 4: 325–336. https://doi.org/10.1016/j.ecolmodel.2008.04.001.

Blanchet, F. G., P. Legendre, and O. Gauthier. 2007. “AEM: Tools to Construct Asymmetric Eigenvector Maps (AEM) Spatial Variables.” http://r-forge.r-project.org/R/?group_id=195.

Blanchet, F. G., P. Legendre, R. Maranger, D. Monti, and P. Pepin. 2011. “Modelling the Effect of Directional Spatial Ecological Processes at Different Scales.” *Oecologia* 166, no. 2: 357–368. https://doi.org/10.1007/s00442-010-1867-y.

Bonada, N., N. Prat, V. H. Resh, and B. Statzner. 2006. “Developments in Aquatic Insect Biomonitoring: A Comparative Analysis of Recent Approaches.” *Annual Review of Entomology* 51, no. 1: 495–523. https://doi.org/10.1146/annurev.ento.51.110104.151124.

Brasseur, M. V., J. Martini, O. Wilfling, et al. 2023. “Exploring Macroinvertebrate Biodiversity in the Dynamic Southern Balkan Stream Network of the Vjosa Using Preservative-Based DNA Metabarcoding.” *Aquatic Sciences* 85, no. 2: 51. https://doi.org/10.1007/s00027-023-00948-w.

Brittain, J. E., and T. J. Eikeland. 1988. “Invertebrate Drift—A Review.” *Hydrobiologia* 166, no. 1: 77–93. https://doi.org/10.1007/BF00017485.

Brown, B. L., and C. M. Swan. 2010. “Dendritic Network Structure Constrains Metacommunity Properties in Riverine Ecosystems.” *Journal of Animal Ecology* 79, no. 3: 571–580. https://doi.org/10.1111/j.1365-2656.2010.01668.x.

Brown, B. L., C. M. Swan, D. A. Auerbach, et al. 2011. “Metacommunity Theory as a Multispecies, Multiscale Framework for Studying the Influence of River Network Structure on Riverine Communities and Ecosystems.” *Journal of the North American Benthological Society* 30, no. 1: 310–327. https://doi.org/10.1899/10-129.1.

Buchner, D., and F. Leese. 2020. “BOLDigger—A Python Package to Identify and Organise Sequences With the Barcode of Life Data Systems.” *Metabarcoding and Metagenomics* 4: e53535. https://doi.org/10.3897/mbmg.4.53535.

Burgers, H. E. R., A. M. Schipper, and A. Jan Hendriks. 2014. “Size Relationships of Water Discharge in Rivers: Scaling of Discharge With Catchment Area, Main-Stem Length and Precipitation: RIVER DISCHARGE SCALING RELATIONSHIPS.” *Hydrological Processes* 28, no. 23: 5769–5775. https://doi.org/10.1002/hyp.10087.

Carstensen, D. W., J. Lessard, B. G. Holt, M. Krabbe Borregaard, and C. Rahbek. 2013. “Introducing the Biogeographic Species Pool.” *Ecography* 36, no. 12: 1310–1318. https://doi.org/10.1111/j.1600-0587.2013.00329.x.

Cid, N., T. Erős, J. Heino, et al. 2022. “From Meta-System Theory to the Sustainable Management of Rivers in the Anthropocene.” *Frontiers in*

Ecology and the Environment 20, no. 1: 49–57. https://doi.org/10.1002/fee.2417.

Cornell, H. V., and S. P. Harrison. 2014. "What Are Species Pools and When Are They Important?" *Annual Review of Ecology, Evolution, and Systematics* 45, no. 1: 45–67. https://doi.org/10.1146/annurev-ecolsys-120213-091759.

Datry, T., S. T. Larned, and K. Tockner. 2014. "Intermittent Rivers: A Challenge for Freshwater Ecology." *Bioscience* 64, no. 3: 229–235. https://doi.org/10.1093/biosci/bit027.

Dray, S., P. Legendre, and P. R. Peres-Neto. 2006. "Spatial Modelling: A Comprehensive Framework for Principal Coordinate Analysis of Neighbour Matrices (PCNM)." *Ecological Modelling* 196, no. 3–4: 483–493. https://doi.org/10.1016/j.ecolmodel.2006.02.015.

Dubart, M., J. Pointier, P. Jarne, and P. David. 2022. "Niche Filtering, Competition and Species Turnover in a Metacommunity of Freshwater Molluscs." *Oikos* 2022, no. 9: e09157. https://doi.org/10.1111/oik.09157.

Durance, I., and S. J. Ormerod. 2007. "Climate Change Effects on Upland Stream Macroinvertebrates Over a 25-Year Period." *Global Change Biology* 13, no. 5: 942–957. https://doi.org/10.1111/j.1365-2486.2007.01340.x.

Dwyer, G. K., B. J. Downes, J. Lancaster, S. P. Rice, L. Slater, and R. E. Lester. 2024. "Spatial Arrangement or Amount? Spatially Variable Oviposition Habitat Can Determine Aquatic Insect Egg Abundance." *Freshwater Biology* 69, no. 12: 1800–1813. https://doi.org/10.1111/fwb.14343.

Elbrecht, V., and F. Leese. 2017. "Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment." *Frontiers in Environmental Science* 5: 11. https://doi.org/10.3389/fenvs.2017.00011.

Emilson, C. E., D. G. Thompson, L. A. Venier, et al. 2017. "DNA Metabarcoding and Morphological Macroinvertebrate Metrics Reveal the Same Changes in Boreal Watersheds Across an Environmental Gradient." *Scientific Reports* 7, no. 1: 12777. https://doi.org/10.1038/s41598-017-13157-x.

Emmons, S. C., Z. G. Compson, M. C. Malish, et al. 2023. "DNA Metabarcoding Captures Different Macroinvertebrate Biodiversity Than Morphological Identification Approaches Across a Continental Scale." *Environmental DNA* 5, no. 6: 1307–1320. https://doi.org/10.1002/edn3.453.

Erba, S., L. Melissano, and A. Buffagni. 2003. "Life Cycles of Baetidae (Insecta: Ephemeroptera) in a North Italian Prealpine Stream." Research Update on Ephemeroptera & Plecoptera, 177–186.

Fuß, T., L. Thuile Bistarelli, R. Ptacnik, and G. A. Singer. 2025. "Niche Partitioning in a Periphyton Metacommunity Peaks at Intermediate Species Richness in Midsized Rivers." *Ecology* 106, no. 1: e4524. https://doi.org/10.1002/ecy.4524.

Fuß, T., L. Thuile Bistarelli, F. Walther, S. Vitecek, L. Talluto, and G. Singer. 2024. "Geodiversity of a European River Network Controls Algal Biodiversity and Function." *Communications Earth & Environment* 5, no. 1: 315. https://doi.org/10.1038/s43247-024-01478-5.

Godsoe, W., J. Franklin, and F. G. Blanchet. 2017. "Effects of Biotic Interactions on Modeled Species' Distribution Can Be Masked by Environmental Gradients." *Ecology and Evolution* 7, no. 2: 654–664. https://doi.org/10.1002/ece3.2657.

Gold, Z., A. O. Shelton, H. R. Casendino, et al. 2023. "Signal and Noise in Metabarcoding Data." *PLoS One* 18, no. 5: e0285674. https://doi.org/10.1371/journal.pone.0285674.

Göthe, E., D. G. Angeler, and L. Sandin. 2013. "Metacommunity Structure in a Small Boreal Stream Network." *Journal of Animal Ecology* 82, no. 2: 449–458. https://doi.org/10.1111/1365-2656.12004.

Graf, W., S. U. Pauls, and S. Vitecek. 2018. "Isoperla Vjosae sp. n., a New Species of the Isoperla Tripartita Group From Albania (Plecoptera: Perlodidae)." *Zootaxa* 4370, no. 2: 171. https://doi.org/10.11646/zootaxa.4370.2.5.

Gravel, D., N. Mouquet, M. Loreau, and F. Guichard. 2010. "Patch Dynamics, Persistence, and Species Coexistence in Metaecosystems." *American Naturalist* 176, no. 3: 289–302. https://doi.org/10.1086/655426.

Hauer, C., K. Skrame, and M. Fuhrmann. 2021. "Hydromorphologial Assessment of the Vjosa River at the Catchment Scale Linking Glacial History and Fluvial Processes." *Catena* 207: 105598. https://doi.org/10.1016/j.catena.2021.105598.

Hauer, C., G. Unfer, W. Graf, P. Leitner, B. Zeiringer, and H. Habersack. 2012. "Hydro-Morphologically Related Variance in Benthic Drift and Its Importance for Numerical Habitat Modelling." *Hydrobiologia* 683, no. 1: 83–108. https://doi.org/10.1007/s10750-011-0942-7.

Heino, J. 2014. "Taxonomic Surrogacy, Numerical Resolution and Responses of Stream Macroinvertebrate Communities to Ecological Gradients: Are the Inferences Transferable Among Regions?" *Ecological Indicators* 36: 186–194. https://doi.org/10.1016/j.ecolind.2013.07.022.

Heino, J., A. S. Melo, L. M. Bini, et al. 2015. "A Comparative Analysis Reveals Weak Relationships Between Ecological Factors and Beta Diversity of Stream Insect Metacommunities at Two Spatial Levels." *Ecology and Evolution* 5, no. 6: 1235–1248. https://doi.org/10.1002/ece3.1439.

Hering, D., A. Borja, J. Carstensen, et al. 2010. "The European Water Framework Directive at the Age of 10: A Critical Review of the Achievements With Recommendations for the Future." *Science of the Total Environment* 408, no. 19: 4007–4019. https://doi.org/10.1016/j.scitotenv.2010.05.031.

Hering, D., A. Buffagni, O. Moog, et al. 2003. "The Development of a System to Assess the Ecological Quality of Streams Based on Macroinvertebrates—Design of the Sampling Programme Within the AQEM Project." *International Review of Hydrobiology* 88, no. 34: 345–361. https://doi.org/10.1002/iroh.200390030.

Humpesch, U. H. 1979. "Autökologische Untersuchungen zum Entwicklungszyklus von Baetis Alpinus (Pict.)." Proc. 2nd Int. Conf. on Ephemeroptera, Krakow., Vol. 1975.

Jacobson, B., and P. R. Peres-Neto. 2010. "Quantifying and Disentangling Dispersal in Metacommunities: How Close Have We Come? How Far Is There to Go?" *Landscape Ecology* 25, no. 4: 495–507. https://doi.org/10.1007/s10980-009-9442-9.

Jacquet, C., C. Moritz, L. Morissette, et al. 2016. "No Complexity–Stability Relationship in Empirical Ecosystems." *Nature Communications* 7: 12573. https://doi.org/10.1038/ncomms12573.

Jacquet, C., F. Munoz, N. Bonada, T. Datry, J. Heino, and F. Jabot. 2022. "Disturbance-Driven Alteration of Patch Connectivity Determines Local Biodiversity Recovery Within Metacommunities." *Ecography* 2022: e06199. https://doi.org/10.1111/ecog.06199.

Jones, J. I., A. Arnold, D. Buchner, et al. 2025. "Sources of Uncertainty in DNA Metabarcoding of Whole Communities: Implications for Its Use in Biomonitoring." *Methods in Ecology and Evolution* 16, no. 8: 1658–1673. https://doi.org/10.1111/2041-210X.70093.

Keck, F., T. Peller, R. Alther, et al. 2025. "The Global Human Impact on Biodiversity." *Nature* 641: 395–400. https://doi.org/10.1038/s41586-025-08752-2.

Kennedy, T. A., C. B. Yackulic, W. F. Cross, P. E. Grams, M. D. Yard, and A. J. Copp. 2014. "The Relation Between Invertebrate Drift and Two Primary Controls, Discharge and Benthic Densities, in a Large Regulated River." *Freshwater Biology* 59, no. 3: 557–572. https://doi.org/10.1111/fwb.12285.

Lai, J., Y. Zou, J. Zhang, and P. R. Peres-Neto. 2022. “Generalizing Hierarchical and Variation Partitioning in Multiple Regression and Canonical Analyses Using the Rdacca. Hp R Package.” *Methods in Ecology and Evolution* 13, no. 4: 782–788. https://doi.org/10.1111/2041-210X.13800.

Leibold, M. A., M. Holyoak, N. Mouquet, et al. 2004. “The Metacommunity Concept: A Framework for Multi-Scale Community Ecology: The Metacommunity Concept.” *Ecology Letters* 7, no. 7: 601–613. https://doi.org/10.1111/j.1461-0248.2004.00608.x.

Leibold, M. A., F. J. Rudolph, F. G. Blanchet, et al. 2022. “The Internal Structure of Metacommunities.” *Oikos* 2022, no. 1: 8618. https://doi.org/10.1111/oik.08618.

Loreau, M., N. Mouquet, and R. D. Holt. 2003. “Meta-Ecosystems: A Theoretical Framework for a Spatial Ecosystem Ecology.” *Ecology Letters* 6, no. 8: 673–679. https://doi.org/10.1046/j.1461-0248.2003.00483.x.

MacArthur, R. H., and E. O. Wilson. 1963. “An Equilibrium Theory of Insular Zoogeography.” *Evolution* 17, no. 4: 373. https://doi.org/10.2307/2407089.

Macher, T.-H., A. J. Beermann, J. Arle, et al. 2025. “Fit for Purpose? Evaluating Benthic Invertebrate DNA Metabarcoding for Ecological Status Class Assessment in Streams Under the Water Framework Directive.” *Water Research* 272: 122987. https://doi.org/10.1016/j.watres.2024.122987.

Martini, J., F. Walther, T. Schenekar, et al. 2023. “The Last Hideout: Abundance Patterns of the Not-Quite-Yet Extinct Mayfly *Prosopistoma pennigerum* in the Albanian Vjosa River Network.” *Insect Conservation and Diversity* 16, no. 2: 285–297. https://doi.org/10.1111/icad.12620.

Martini, J., and J. Waringer. 2021. “Dynamic Microhabitat Shifts in Space and Time of Caddisfly Larvae (Insecta: Trichoptera) in a First-Order Calcareous Mountain Stream.” *Biologia* 76, no. 9: 2527–2541. https://doi.org/10.1007/s11756-021-00741-w.

McCann, K. S., and N. Rooney. 2009. “The More Food Webs Change, the More They Stay the Same.” *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 364, no. 1524: 1789–1801. https://doi.org/10.1098/rstb.2008.0273.

Nakazawa, T. 2015. “Ontogenetic Niche Shifts Matter in Community Ecology: A Review and Future Perspectives.” *Population Ecology* 57, no. 2: 347–354. https://doi.org/10.1007/s10144-014-0448-z.

Oksanen, J., G. L. Simpson, M. Friendly, et al. 2020. “Vegan: Community Ecology Package. R Package Version 2.5–7.” https://CRAN.R-project.org/package=vegan.

Pichler, M., S. Creer, A. Martínez, D. Fontaneto, W. Renema, and J. Macher. 2025. “Metacommunity Theory and Metabarcoding Reveal the Environmental, Spatial and Biotic Drivers of Meiofaunal Communities in Sandy Beaches.” *Molecular Ecology* 34, no. 8: e17733. https://doi.org/10.1111/mec.17733.

Pichler, M., and F. Hartig. 2021. “A New Joint Species Distribution Model for Faster and More Accurate Inference of Species Associations From Big Community Data.” *Methods in Ecology and Evolution* 12, no. 11: 2159–2173. https://doi.org/10.1111/2041-210X.13687.

Poff, N. L., J. D. Allan, M. B. Bain, et al. 1997. “The Natural Flow Regime.” *Bioscience* 47, no. 11: 769–784. https://doi.org/10.2307/1313099.

Pollice, A., G. Jona-Lasinio, M. Gaglio, F. G. Blanchet, and E. A. Fano. 2020. “Modelling the Effect of Directional Spatial Ecological Processes for a River Network in Northern Italy.” *Ecological Indicators* 112: 106144. https://doi.org/10.1016/j.ecolind.2020.106144.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/.

Ratnasingham, S., and P. D. N. Hebert. 2007. “BARCODING: Bold: The Barcode of Life Data System.” *Molecular Ecology Notes* 7, no. 3: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x.

Remmel, N., D. Buchner, J. Enss, et al. 2024. “DNA Metabarcoding and Morphological Identification Reveal Similar Richness, Taxonomic Composition and Body Size Patterns Among Flying Insect Communities.” *Insect Conservation and Diversity* 17, no. 3: 449–463. https://doi.org/10.1111/icad.12710.

Ricklefs, R. E. 1987. “Community Diversity: Relative Roles of Local and Regional Processes.” *Science* 235, no. 4785: 167–171. https://doi.org/10.1126/science.235.4785.167.

Šamulková, M., P. Beracko, P. Macko, et al. 2025. “Evaluating the Potential of DNA Metabarcoding for Ecological Status Assessment Under the Water Framework Directive: A Case Study on Benthic Invertebrates From Western Carpathian Streams.” *Metabarcoding and Metagenomics* 9: e163640. https://doi.org/10.3897/mbmg.9.163640.

Sand, K., and J. E. Brittain. 2009. “Life Cycle Shifts in Baetis Rhodani (Ephemeroptera) in the Norwegian Mountains.” *Aquatic Insects* 31, no. sup1: 283–291. https://doi.org/10.1080/01650420902732362.

Schiemer, F., S. Beqiraj, A. Drescher, et al. 2020. “The Vjosa River Corridor: A Model of Natural Hydro-Morphodynamics and a Hotspot of Highly Threatened Ecosystems of European Significance.” *Landscape Ecology* 35, no. 4: 953–968. https://doi.org/10.1007/s10980-020-00993-y.

Scholl, E. A., W. F. Cross, C. S. Guy, A. J. Dutton, and J. R. Junker. 2023. “Landscape Diversity Promotes Stable Food-Web Architectures in Large Rivers.” *Ecology Letters* 26, no. 10: 1740–1751. https://doi.org/10.1111/ele.14289.

Schwingshackl, T., J. Martini, M. Yegon, G. Singer, and S. Vitecek. 2024. “Tiny but Mighty Mayfly—Probing Prosopistoma Pennigerum (Müller 1785) as the Flagship Species for the Vjosa Wild River National Park.” *Journal of Insect Conservation* 28: 1185–1198. https://doi.org/10.1007/s10841-024-00616-w.

Serrana, J. M., Y. Miyake, M. Gamboa, and K. Watanabe. 2019. “Comparison of DNA Metabarcoding and Morphological Identification for Stream Macroinvertebrate Biodiversity Assessment and Monitoring.” *Ecological Indicators* 101: 963–972. https://doi.org/10.1016/j.ecolind.2019.02.008.

Singer, A., J. M. J. Travis, and K. Johst. 2013. “Interspecific Interactions Affect Species and Community Responses to Climate Shifts.” *Oikos* 122, no. 3: 358–366. https://doi.org/10.1111/j.1600-0706.2012.20465.x.

Stephan, P., B. Bramon Mora, and J. M. Alexander. 2021. “Positive Species Interactions Shape Species' Range Limits.” *Oikos* 130, no. 10: 1611–1625. https://doi.org/10.1111/oik.08146.

Stoffers, T., F. Altermatt, D. Baldan, et al. 2024. “Reviving Europe's Rivers: Seven Challenges in the Implementation of the Nature Restoration Law to Restore Free-Flowing Rivers.” *WIREs Water* 11, no. 3: e1717. https://doi.org/10.1002/wat2.1717.

Sunnucks, P., and D. F. Hales. 1996. “Numerous Transposed Sequences of Mitochondrial Cytochrome Oxidase I-II in Aphids of the Genus Sitobion (Hemiptera: Aphididae).” *Molecular Biology and Evolution* 13, no. 3: 510–524. https://doi.org/10.1093/oxfordjournals.molbev.a025612.

Talluto, L. 2020. “WatershedTools: An R Package for the Spatial Analysis of Watersheds.” https://github.com/mtalluto/WatershedTools.

Talluto, L., I. Boulangeat, S. Vissault, W. Thuiller, and D. Gravel. 2017. “Extinction Debt and Colonization Credit Delay Range Shifts of Eastern North American Trees.” *Nature Ecology & Evolution* 1, no. 7: 182. https://doi.org/10.1038/s41559-017-0182.

Talluto, L., R. Del Campo, E. Estévez, F. Altermatt, T. Datry, and G. Singer. 2024. “Towards (Better) Fluvial Meta-Ecosystem Ecology: A Research Perspective.” *npj Biodiversity* 3, no. 1: 3. https://doi.org/10.1038/s44185-023-00036-0.

Thuile Bistarelli, L., T. Fuß, F. Walther, et al. 2024. "Strong Large-Scale Structure–Function Coupling in Benthic Bacteria Is Mediated by Algae in a Geodiverse River Network." *Limnology and Oceanography* 69: 2624–2638. https://doi.org/10.1002/lno.12690.

Tickner, D., J. J. Opperman, R. Abell, et al. 2020. "Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan." *Bioscience* 70, no. 4: 330–342. https://doi.org/10.1093/biosci/biaa002.

Tonkin, J. D., F. Altermatt, D. S. Finn, et al. 2018. "The Role of Dispersal in River Network Metacommunities: Patterns, Processes, and Pathways." *Freshwater Biology* 63, no. 1: 141–163. https://doi.org/10.1111/fwb.13037.

Vanschoenwinkel, B., C. De Vries, M. Seaman, and L. Brendonck. 2007. "The Role of Metacommunity Processes in Shaping Invertebrate Rock Pool Communities Along a Dispersal Gradient." *Oikos* 116, no. 8: 1255–1266. https://doi.org/10.1111/j.0030-1299.2007.15860.x.

Venail, P. A., R. C. MacLean, T. Bouvier, M. A. Brockhurst, M. E. Hochberg, and N. Mouquet. 2008. "Diversity and Productivity Peak at Intermediate Dispersal Rate in Evolving Metacommunities." *Nature* 452, no. 7184: 210–214. https://doi.org/10.1038/nature06554.

Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, et al. 2010. "Global Threats to Human Water Security and River Biodiversity." *Nature* 467, no. 7315: 555–561. https://doi.org/10.1038/nature09440.

Ward, C. A., T. D. Tunney, and K. S. McCann. 2023. "Managing Aquatic Habitat Structure for Resilient Trophic Interactions." *Ecological Applications* 33, no. 3: e2814. https://doi.org/10.1002/eap.2814.

Ward, J. V. 1985. "Thermal Characteristics of Running Waters." *Hydrobiologia* 125, no. 1: 31–46. https://doi.org/10.1007/BF00045924.

Waringer, J., J. Martini, and S. Vitecek. 2020. "A Remarkable Caddisfly From the Vjosa River Catchment: *Thremma anomalum* McLachlan 1876." *Acta ZooBot Austria* 157: 261–274.

Warton, D. I., F. G. Blanchet, R. B. O'Hara, et al. 2015. "So Many Variables: Joint Modeling in Community Ecology." *Trends in Ecology & Evolution* 30, no. 12: 766–779. https://doi.org/10.1016/j.tree.2015.09.007.

Weiss, M., and F. Leese. 2016. "Widely Distributed and Regionally Isolated! Drivers of Genetic Structure in Gammarus Fossarum in a Human-Impacted Landscape." *BMC Evolutionary Biology* 16, no. 1: 153. https://doi.org/10.1186/s12862-016-0723-z.

Wisz, M. S., J. Pottier, W. D. Kissling, et al. 2013. "The Role of Biotic Interactions in Shaping Distributions and Realised Assemblages of Species: Implications for Species Distribution Modelling." *Biological Reviews* 88, no. 1: 15–30. https://doi.org/10.1111/j.1469-185X.2012.00235.x.

Yamaguchi, R. 2022. "Intermediate Dispersal Hypothesis of Species Diversity: New Insights." *Ecological Research* 37, no. 3: 301–315. https://doi.org/10.1111/1440-1703.12313.

Zizka, V. M. A., V. Elbrecht, J. Macher, and F. Leese. 2019. "Assessing the Influence of Sample Tagging and Library Preparation on DNA Metabarcoding." *Molecular Ecology Resources* 19, no. 4: 893–899. https://doi.org/10.1111/1755-0998.13018.

Zou, H.-X., and V. H. W. Rudolf. 2023. "Bridging Theory and Experiments of Priority Effects." *Trends in Ecology & Evolution* 38, no. 12: 1203–1216. https://doi.org/10.1016/j.tree.2023.08.001.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ece373307-sup-0001-Supinfo01.docx.